ZOOM

Next Generation Sequencing Software

User's Manual
Version 1.5

from the makers of PatternHunter
Bioinformatics Solutions Inc.

BIOINFORMATICS SOLUTIONS INC

ZOOM Studio User's Manual

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or suggestions for improvement.

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Chapter

1

Introduction

1. Introduction to ZOOM

ZOOM (Zillions Of Oligos Mapped) is designed to map millions of short reads, produced by next-generation sequencing technology, back to the reference genome, and carry out post-analysis in a user-friendly way. Based on a newly designed multiple spaced seeds theory, ZOOM guarantees great mapping accuracy with unparalleled speed. Both single and paired-end reads of various lengths from 12bp to 240bp can be handled. Any number of mismatches and one insertion/deletion of various lengths between the read and its target region on the reference sequence are allowed. Uniquely mapped results or best (top N) results for each read will be reported, according to the minimal mismatches and indel length between the read and its target positions.

ZOOM supports both Illumina/Solexa and ABI SOLiD instruments. For Illumina/Solexa data, quality scores generated by the sequencer for each of the short sequenced reads can be incorporated to reduce ambiguity of read mapping. For ABI SOLiD data, ZOOM directly aligns a color space read to a base space reference sequence. ZOOM is therefore able to differentiate a true polymorphism on the base space from the sequencing errors on the color space, and automatically corrects sequencing errors during the mapping process. Reads in color space will be decoded into base space, with both sequencing errors on color space and true polymorphisms to their target region on the reference genome marked, respectively.

Terminology and Abbreviations Glossary

Base space: reads represented in the alphabet of nucleotides *{A, C, G, T, N}*, such as *ACGTAAA*

BSI (Bioinformatics Solutions Inc.): the maker of PEAKS, PatternHunter, RAPTOR, ZOOM and other fine bioinformatics software

Color space: also called di-base alphabet. This is the data format produced by the ABI SOLiD sequencer. Reads are represented as colors, in the way that two adjacent nucleotides are encoded by one color letter, represented as *{0, 1, 2, 3}*. The convert from base space to color space uses the following table:

	A	C	G	T
A	0	1	2	3
C	1	0	3	2
G	2	3	0	1
T	3	2	1	0

Coverage: the number of reads that one segment/area of the reference sequence is sequenced. It also means the number of reads mapped back to one position or one area of the reference sequence.

Edit distance: the summation of the number of mismatches and the lengths of indels

Hamming distance: the number of mismatches between a read and its target region on the reference sequence

Indel: insertion and deletion mutations

Mismatch: A mismatch occurs when the nucleotide base from the read and the reference sequence are different, or when either of the sequences has an '*N*' at that position. If the sequencing qualities are also used, the mismatches occurring at low quality sites (determined by a quality threshold) will be ignored.

Multiple spaced seeds: Multiple spaced seeds, which further enhance the sensitivity, are several spaced seeds optimized simultaneously against a given level of similarity. PatternHunter II using multiple spaced seeds would approach the sensitivity of the Smith-Waterman algorithm while gaining Blastn speed.

Oligos: oligonucleotides, short DNA or RNA sequences

Optimal spaced seed: a novel idea proposed first in PatternHunter to enhance both sensitivity and speed of filtering in the pairwise homology search process. Compared to a consecutive seed which requires the query sequence and the target sequence to share a sequence block of same nucleotides, optimal spaced seed requires only designated positions to be the same. The strategy was proven in PatternHunter to enhance sensitivity and speed greatly when compared to BLAST.

Quality score: the quality or confidence score of each nucleotide sequenced. It is a hint of the probability of this position is correctly sequenced.

Reference offset: the leftmost position where a read is mapped onto the reference sequence.

Paired-end reads: two reads sequenced from both ends of the DNA fragment. The paired-end reads from the same region of the reference sequence are expected to be located on the same chain and separated by a known distance range. The orientation and distance limit help to locate unambiguous reads. They are also helpful in finding insertion/deletion and structural variations.

100% Sensitivity: the full capacity to find all target regions within user-defined mismatches on the reference sequence for each read

SNP: Single Nucleotide Polymorphism. SNP is a DNA sequence variation occurring when a single nucleotide — A, T, C, or G — in the genome (or other shared sequence) differs between members of a species (or between paired chromosomes in an individual).

Single-end reads: reads that were sequenced separately

Target region: reference sequence segment where the read is mapped

Uniquely mapped read: Each read might be mapped to multiple target regions in the reference sequence. The best mapping results of one read are the ones with smallest edit distance, or in the case of an equal edit distance, the shortest indel length (under the assumption that indels are less probable than mutations). If there is only one such best mapping result for the read, this is a uniquely mapped read. Otherwise, if there are multiple such mappings, the read will be considered ambiguously mapped.

For example, let A and B be two reference positions:

- If a read can be mapped to position A and position B on the reference genome, with two mismatches for A, and one mismatch for B, then B is reported as the unique mapping position for this read.
- If both A and B contain two mismatches, then this read is not reported
- If there are two mismatches and an indel of length one for A, one mismatch and an indel of length two for B, then A is reported.
- If there are two mismatches for both A and B, an indel of length one for A, an indel of length two for B, then A is reported.
- If, there are two mismatches and an indel of length one for both A and B, then this read is not reported.

The depth of mapped reads / Coverage: The amount of mapped reads covering the position of the reference sequence is called the depth of mapped reads on the position or the coverage of the position on the reference sequence.

ZOOM: Zillions Of Oligos Mapped, a next generation sequencing analysis tool

Chapter

2

Installation

2. Getting started with ZOOM

2.1 Package contents

The ZOOM package should contain:

- This manual (hardcopy and/or electronic version)
- ZOOM software

2.2 System requirements

ZOOM will run on most platforms with the following requirements:

- Processor: Equivalent or superior processing power to an Intel™ Pentium 4 Processor 1.6GHz
- Memory: 2 GB memory (8 GB RAM is recommended for processing large data set)
- Operation System: Microsoft Windows XP or above, and/or 64bit Unix/Linux operation system
- Display: 800 pixels by 600 pixels minimal

2.3 Instrumentation

ZOOM will work with both single-end reads and paired-end reads of length ranging from 12bp to 240bp from the following next generation sequencing instruments:

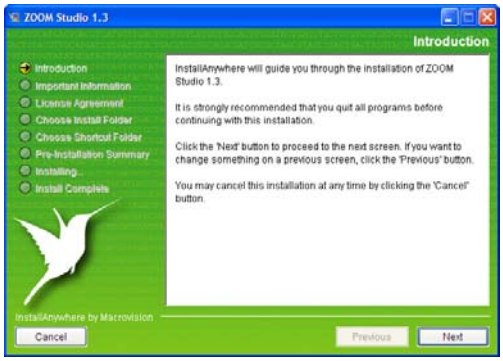
- Illumina/Solexa: *_seq.txt and *_prb.txt, *.fastq
- Applied Biosystems Inc. SOLiD: *.csfasta, *_QV.qual, *.fastq

2.4 Install ZOOM Studio

Note: If you already have an older version of ZOOM installed on your system, please uninstall it before proceeding.

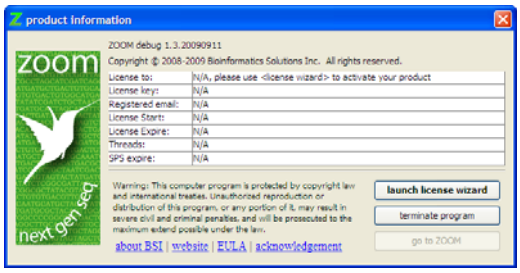
1. Close all programs that are currently running.
2. Insert the ZOOM disc into the CD-ROM/DVD-ROM drive. If loading ZOOM via the download link, skip to step 4, after downloading and running the file.

3. Auto-run should automatically load the installation software. If it does not, find the CD-ROM drive and open it to access the disc. Click on the autorun.exe. (On Linux system, click ZOOMsetup.bin)


ZOOM Studio 1.3
Introduction
Introduction
Important Information
License Agreement
Choose Install Folder
Choose Shortcut Folder
Pre-Installation Summary
Installing...
Install Complete
InstallAnywhere will guide you through the installation of ZOOM Studio 1.3.
It is strongly recommended that you quit all programs before continuing with this installation.
Click the 'Next' button to proceed to the next screen. If you want to change something on a previous screen, click the 'Previous' button.
You may cancel this installation at any time by clicking the 'Cancel' button.
InstallAnywhere by Macrovision
Cancel
Previous
Next

4. A menu screen will appear. Select the top item “ZOOM Installation”. The installation utility will begin the install. Wait while it does so. When the “ZOOM Studio” installation dialogue appears, click the “Next” button.
5. Basic system requirements will be presented. “Click Next”.
6. Read the license agreement. If you agree with it, change the radio button at the bottom to select “I accept the terms of the License Agreement” and click “Next”.
7. Choose the folder/directory in which you would like to install ZOOM. To change the default location press the “Choose…” button to browse your system and make a selection, or type a folder name in the textbox. Please avoid installing ZOOM in the “Program Files” directory as well as in any directory for which the ZOOM user will not have write-permissions. Click “Next”.
8. Choose where you would like to place icons for ZOOM Studio. The default will put these icons in the programs section of your start menu. A common user preference is on the desktop. Click “Next”.
9. Review the choices you have made. You can click “Previous” if you would like to make any changes or click “Next” if those choices are correct.
10. ZOOM Studio will now install on your system. You may cancel at any time by pressing the “Cancel” button in the lower left corner.
11. When the installation is complete, click “Done”. The ZOOM Studio menu screen should still be open. You may view movies and materials from here. To access this menu at a future date, simply insert the disc in your CD-ROM drive.

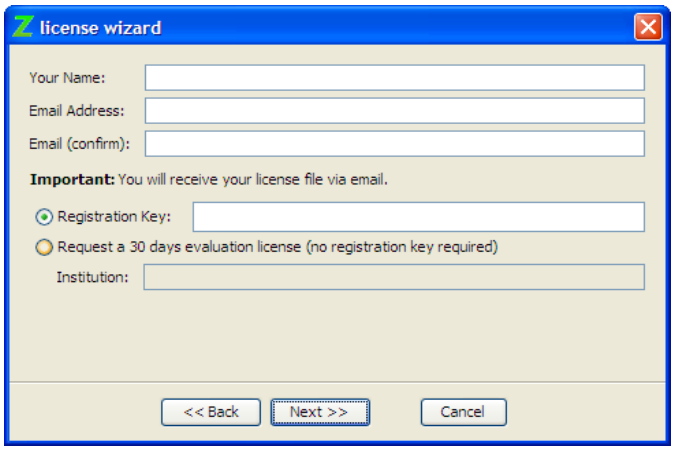
2.5 Registering ZOOM



The first time ZOOM is run, the "About dialogue" containing license wizard will appear automatically. Click "launch license wizard" button to register your copy of ZOOM :

Registration Instructions (Internet Connection)

1. Select "Request a license file (has Internet connection)" and click "Next".
2. The following window will appear:



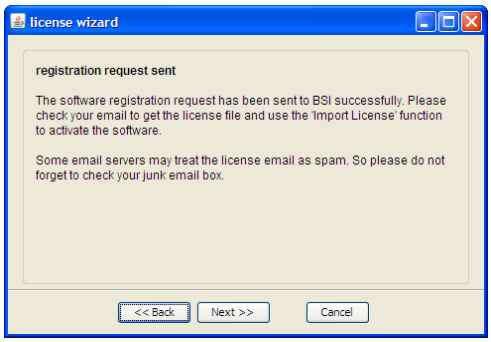
If you have purchased ZOOM and have a registration key, select "Registration Key". Enter your registration key as well as your name and email address and click "Next".

OR

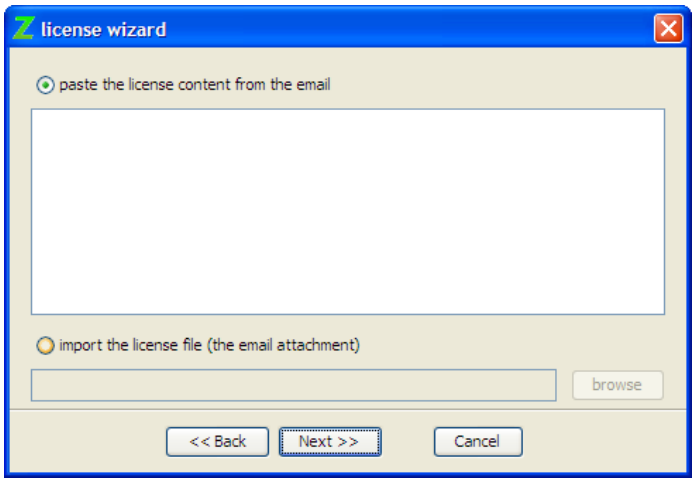
If you are trying a demo of ZOOM and do not have a registration key, select "Request a 30 days evaluation license (No registration key required). Enter your name, email address, as well as your institution. Click "Next".

3. The following window will appear:

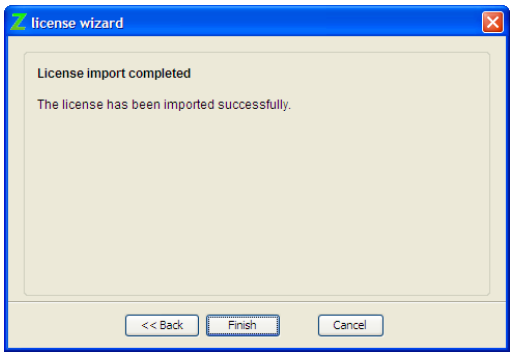
An automated BSI service will generate the license file (license.lcs) and email it to the provided email account from the License Wizard. You can either save the attachment to a local directory or copy the content between '===>' and '<===' in the email. Click "Next".


license wizard
registration request sent
The software registration request has been sent to BSI successfully. Please check your email to get the license file and use the 'Import License' function to activate the software.
Some email servers may treat the license email as spam. So please do not forget to check your junk email box.
<< Back
Next >>
Cancel

4. The following window will appear:



Select “paste the license content from the email” to paste the license information between '===>' and '<===' in the email or select “import the license file (the email attachment) and browse to locate the license file (license.lcs). Click “Next”.

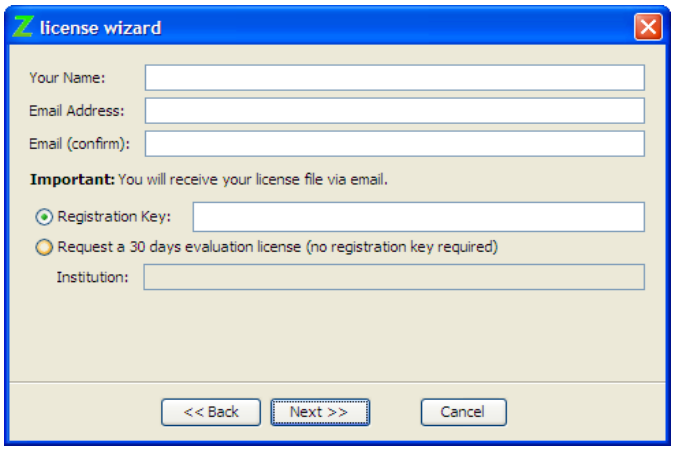


5. The following window will open:

Click “Finish” if you receive a message that the license has been imported successfully.

Registration Instructions (No Internet Connection)

1. Select “Request license file (without Internet connection)” and click “Next” twice.
2. The following window will open:

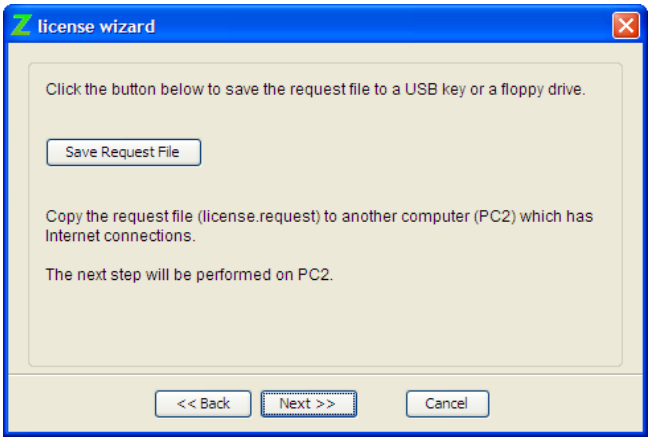


If you have purchased ZOOM and have a registration key, select “Registration Key”. Enter your registration key as well as your name and email address and click “Next”.

OR

If you are trying a demo of ZOOM and do not have a registration key, select “Request a 30 days evaluation license (No registration key required). Enter your name, email address, as well as your institution. Click “Next”.

3. The following window will appear:


license wizard
Click the button below to save the request file to a USB key or a floppy drive.
Save Request File
Copy the request file (license.request) to another computer (PC2) which has Internet connections.
The next step will be performed on PC2.
<< Back
Next >>
Cancel

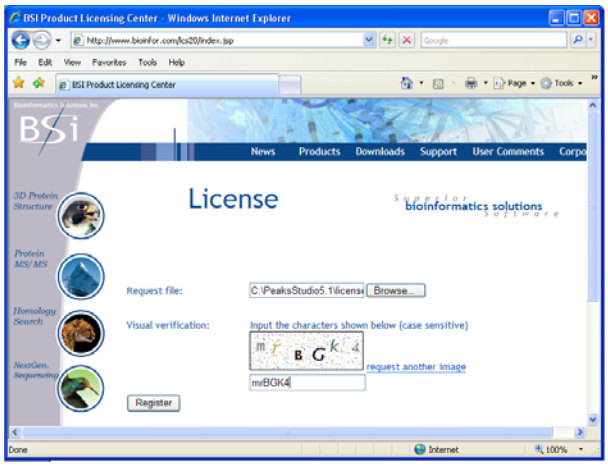
Select the "Save Request File" button to save license.request to your computer (PC1). Click "Next".

4. Transfer the license.request file from PC1 onto a computer with an Internet connection (PC2) using a USB key or a removable storage device. On PC2, go to http://www.bioinfor.com/lcs20/

BSI Product Licensing Center - Windows Internet Explorer
License
bioinformatics solutions
I have the license request file. I want to register the software.
I have license file. I want to update my license.
Next >

5. Select "I have the license request file. I want to register the software" and click "Next".

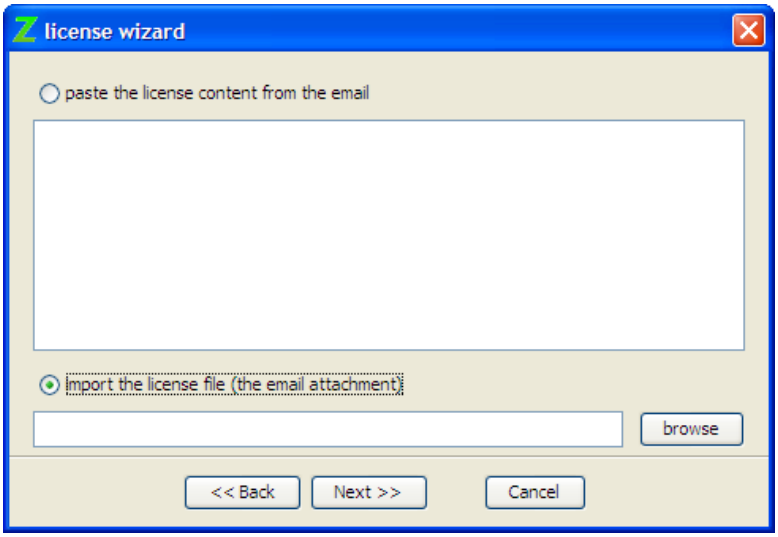
6. The following window will appear:



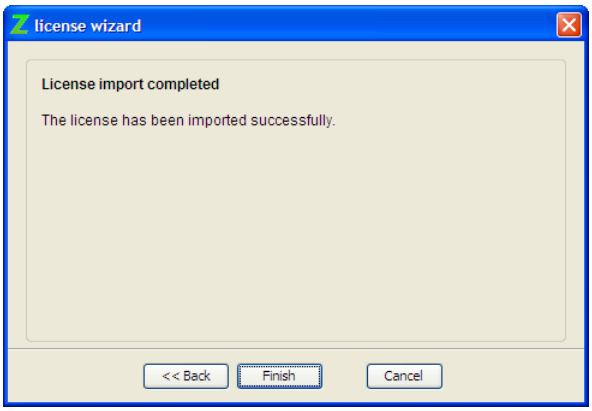
Click the "Browse" button to select the license.request file, type in the visual verification code and click "Next".

7. After the license email is received on PC2, save the attachment, license.lcs, as is and copy the file to PC1. If you do not receive the license.lcs file in your inbox, please check your junk mail folder.

8. In the license wizard on PC1, click the 'browse' button below to select the license.lcs file and click “Next”.

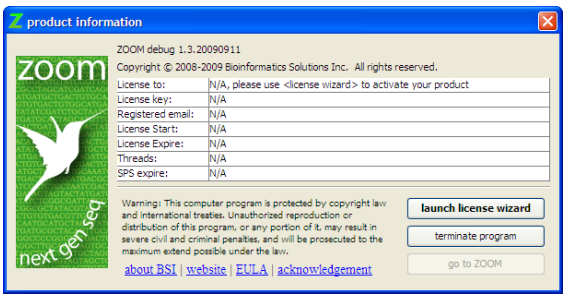


9. Click “Finish” if you receive a message that the license has been imported successfully.



Re-registration Instructions

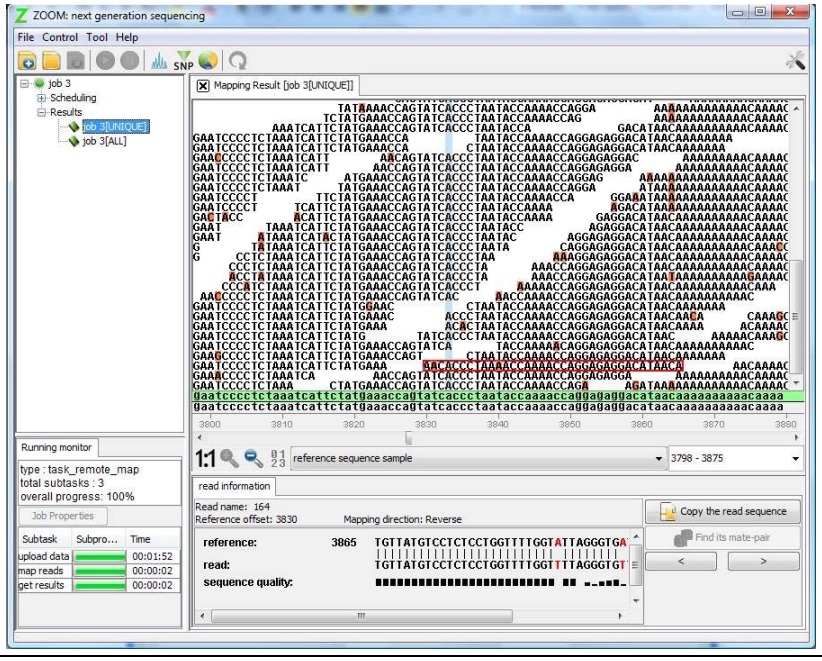
Re-registering ZOOM may be necessary if your license has expired or if you wish to update the license. You will need to obtain a new registration key from BSI. Once you have obtained this new key, select "About" from the Help menu. The "product information" dialogue box will appear:


product information
ZOOM debug 1.3.20090911
Copyright © 2008-2009 Bioinformatics Solutions Inc. All rights reserved.
License to: N/A, please use <license wizard> to activate your product
License key: N/A
Registered email: N/A
License Start: N/A
License Expire: N/A
Threads: N/A
SPS expire: N/A
Warning: This computer program is protected by copyright law and international treaties. Unauthorized reproduction or distribution of this program, or any portion of it, may result in severe civil and criminal penalties, and will be prosecuted to the maximum extend possible under the law.
launch license wizard
terminate program
go to ZOOM
about BSI | website | EULA | acknowledgement

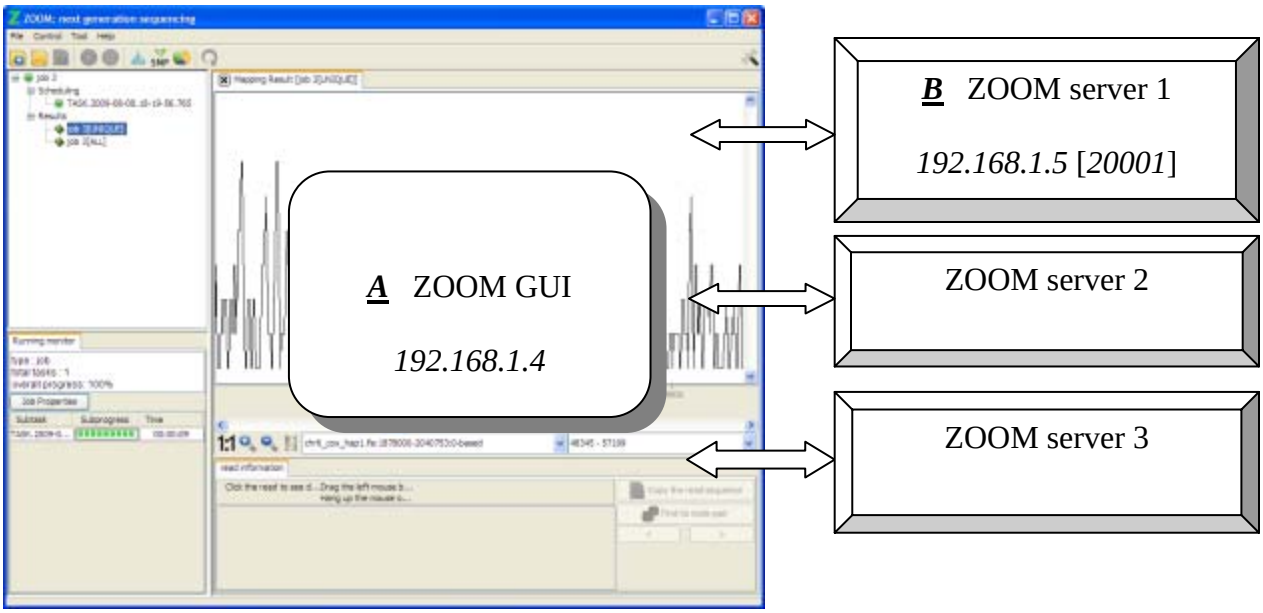
Click the "launch license wizard" button to continue. Then follow the instructions listed above for registering ZOOM Studio.

2.6 Set up your working environment

The ZOOM Studio works in client-server mode. The following graphical user interface (called "ZOOM GUI") is the main work space for you to load your data, submit them to server(s) for computational tasks, monitor the working progress, and view the analysis results:



ZOOM GUI relies on one or more components to perform the actual time-consuming computational tasks. These components are called "ZOOM servers" (or "servers" for short in this manual), which do not necessarily have to reside in the same machine as the ZOOM GUI. Generally, the more ZOOM servers that are used, the faster and less time you will need to process data, illustrated below:



By default, ZOOM Studio already provides and started a local ZOOM server, so you can start your work right now without more advanced settings (You can verify the existence of local ZOOM server by clicking the icon).

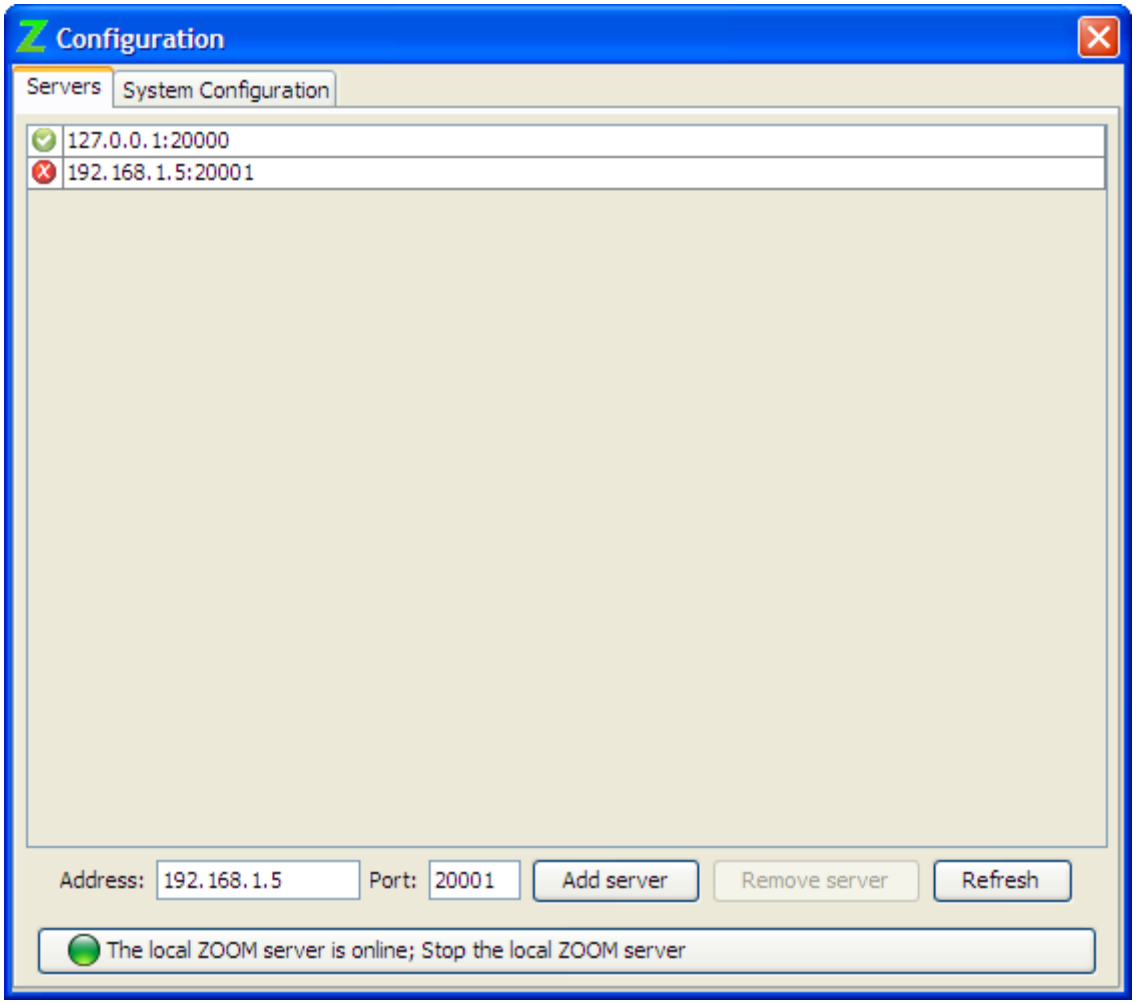
NOTICE: For Windows users, the built-in server has limited processing capability, and we therefore strongly recommend the user to use the LINUX ZOOM servers instead.

If the user needs more advanced features such as starting multiple servers or starting a remote ZOOM server, or utilizing multiple cores of modern CPUs, please follow these steps to add a ZOOM server manually. The user is required to configure both the client side (computer A, where ZOOM GUI is) and the server side (computer B, where ZOOM server is).

Suppose the ZOOM GUI is running on computer A with IP address *192.168.1.4*, and the ZOOM server is going to run on the port *20001* on the computer B with IP address *192.168.1.5*).

Configuration of the client side (Computer A)

1. On computer A, in the ZOOM GUI click the icon on the right side of the toolbar to launch the configuration dialog.
2. Input *192.168.1.5* in the address box and *20001* in the port box, then click the add server button (the user may wish to remove the existing servers first). The new ZOOM server appears in the list but is deactivated (on the left), because it has not been launched on computer B yet.



3. Close the dialog.

Configuration of the server side (Computer B)

Important: each copy of ZOOM server requires its own directory to run, and multiple servers should NEVER be launched within same directory.

Computer B can have either a Windows or LINUX platform, and the users should choose the appropriate distribution of server binary file for their system. If B is Windows platform, the ZOOM server file is called zoomsrv.exe (together with supporting pthreadGC2.dll and mingwm10.dll), and if B has LINUX platform, the ZOOM server file is called ZOOM. Copy the proper ZOOM server file in the ZOOM package to computer B.

For Windows platform:

1. On computer B, create a new directory and transfer zoomsrv.exe, pthreadGC2.dll, mingwm10.dll, start_server.bat into it. You should always create a new directory for each copy of ZOOM server.
2. Use file editor (such as Notepad) to open start_server.bat , search for corresponding lines and change as follow:
3. Tell the ZOOM server where the ZOOM GUI is:

```
set ZOOMGUI=192.168.1.4
```

4. Specify the port the ZOOM server is going to use:

```
set SERVER_PORT=20001
```

5. Specify how many cores the ZOOM server will use (assuming a quad-core CPU):

```
set MAX_CLIENT=4
```

6. Execute start_server.bat to start up the ZOOM server.

For Linux platform:

1. On computer B, create a new directory and transfer ZOOM, start_server.sh into it. You should always create a new directory for each copy of ZOOM server.
2. Use file editor (such as vim) to open start_server.sh, search for corresponding lines and change as follow:

3. Tell the ZOOM server where the ZOOM GUI is:

```
export ZOOMGUI=192.168.1.4
```

4. Specify the port the ZOOM server is going to use:

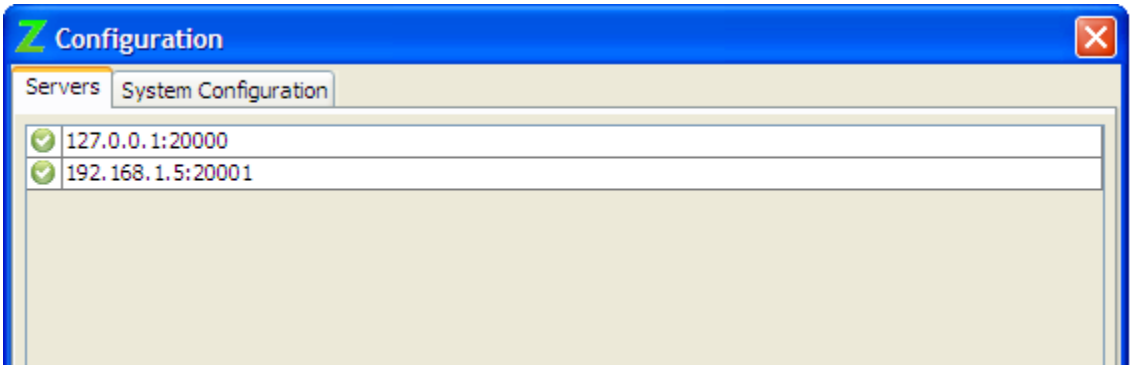
```
export SERVER_PORT=20001
```

5. Specify how many cores the ZOOM server will use (assuming a quad-core CPU):

```
export MAX_CLIENT=4
```

6. Execute start_server.bat to start up the ZOOM server.

Back on computer A, in the ZOOM GUI click the icon again to verify that the newly added ZOOM server is activated. If the icon turns to , then the new server has been correctly launched.

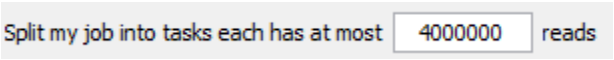


The user can repeat these steps to start up more servers on different ports on computer B, or start up more servers on other computers or even different platforms.

Setup job batch size

ZOOM will split large reads data into several small data files. According to the number of CPUs you assigned, these small data will automatically be scheduled to run in parallel on multiple CPUs. To fit the multiple small data files in the RAM of server, you'd better modify the size of the split files according to the RAM per CPU can use. For example, if you have a server with 8G RAM and you have set MAX_CLIENT=4 (i.e. four tasks can be run in parallel), then the RAM

each CPU can use is 8G / 4 = 2G. The default data size is 4 million reads per small file, which is good for 2G RAM per CPU. If the RAM per CPU on your server is smaller or larger, click , choose “System Configuration” and decrease or increase the batch file size.



We use the RAM per CPU rather than the total RAM of the server as the criteria to decide the amount of reads of each task is because different servers might have different architectures. For example, some architecture is multiple CPUs sharing the same RAM, while others are multiple CPUs which have their own RAMs.

2.7 Command line usage of ZOOM

Starting from version 1.3.0, computational tasks are carried out and completed between ZOOM GUI and ZOOM server cooperatively, but the users can still use the ZOOM server as a command line tool.

Chapter

3

Quick Start

3. Quick Start to Use ZOOM

This section of the manual will walk you through most of the basic functionality of ZOOM. After completing this section you will see how easy it is to map a huge amount of reads with automatic scheduling, view mapping results and find SNPs and short Insertions/Deletions on both single-end reads and paired-end reads for both Illumina/Solexa instrument and ABI SOLiD instrument.

3.1 Sample Data

ZOOM provides two sets of sample data in the "Sample_Data" directory. The "Solexa" directory contains an Illumina/Solexa test data set and the "SOLiD" directory has ABI SOLiD test data set.

1. In the Solexa directory, there are two directories:

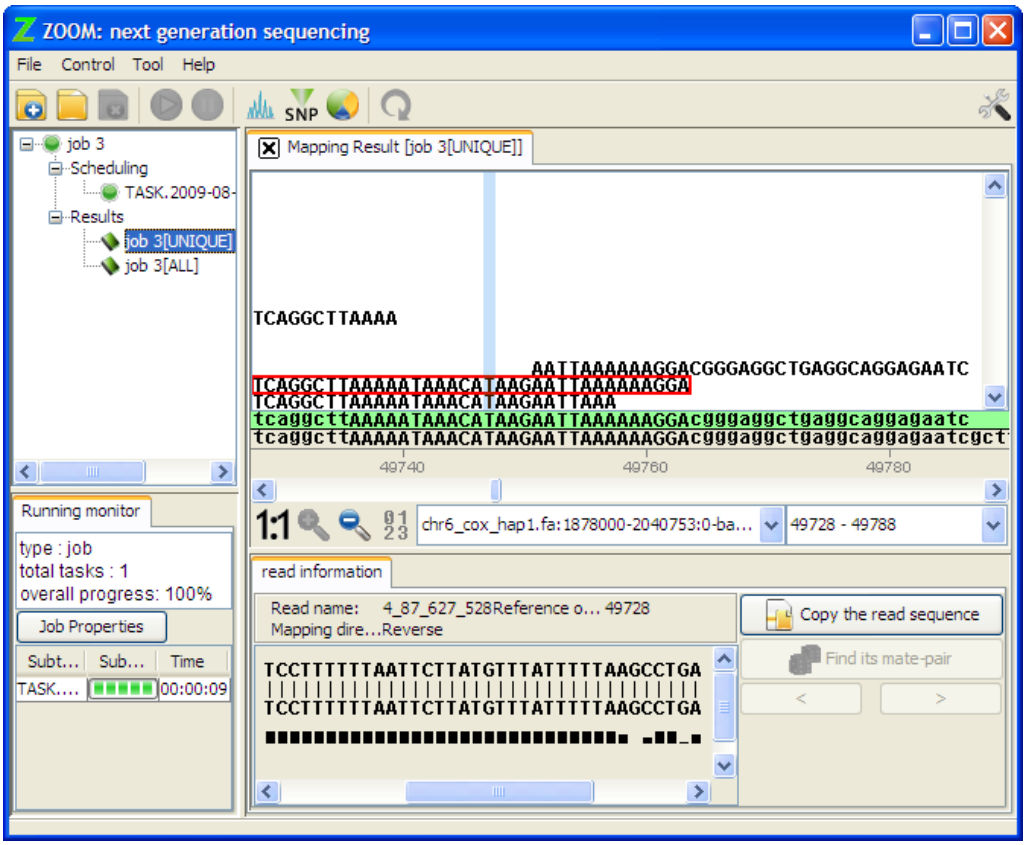
   - "single_end" directory: "read.fastq" and "reference.fa";
   - "paired_end" directory: "read_1.fastq", "read_2.fastq" and "reference.fa";

2. In the SOLiD directory, there are two directories:

   - "single_end" directory: "read.fastq" and "reference.fa";
   - "paired_end" directory: "read_F3.csfasta", "read_F3_QV.qual" and "reference.fa";

3.2 The main windows of ZOOM

The following picture shows the main windows of ZOOM:

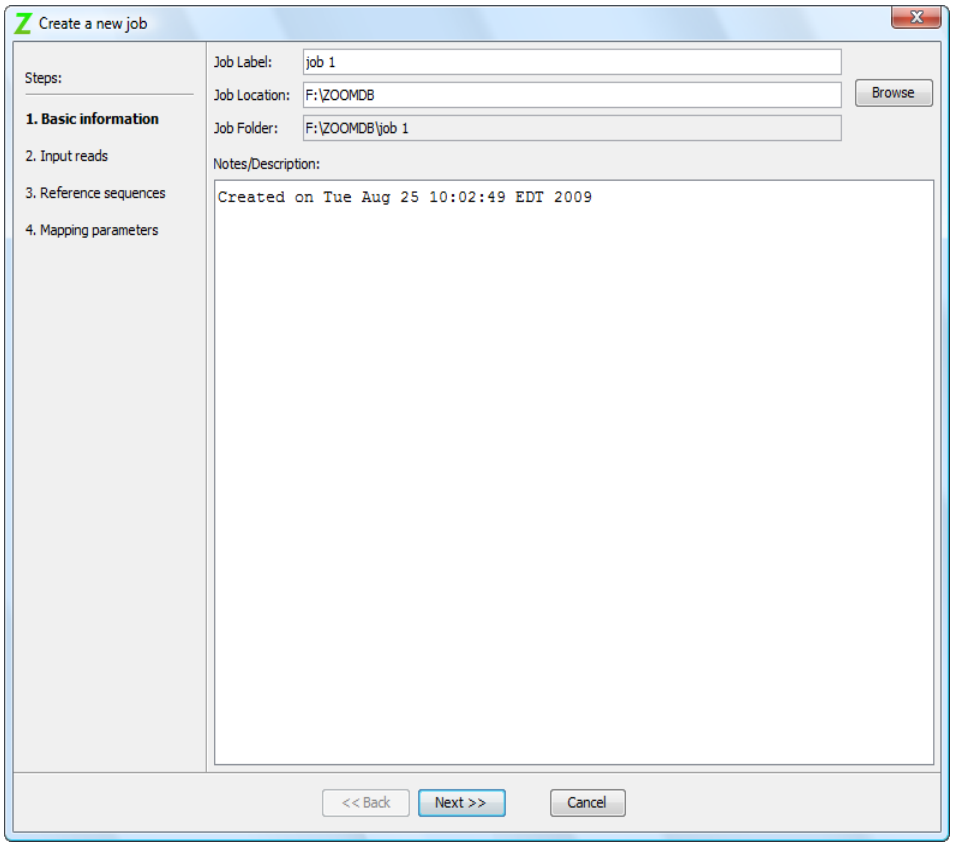


3.3 Set up your working environment

ZOOM works in a client-server mode. By default, ZOOM will launch a server in the local computer. Let's use the default configuration in this quick start section. If you want to use different servers or multiple CPUs on multiple servers, refer to the "Set up your working environment" section in Chapter 2 to configure the ZOOM GUI client and ZOOM server properly.

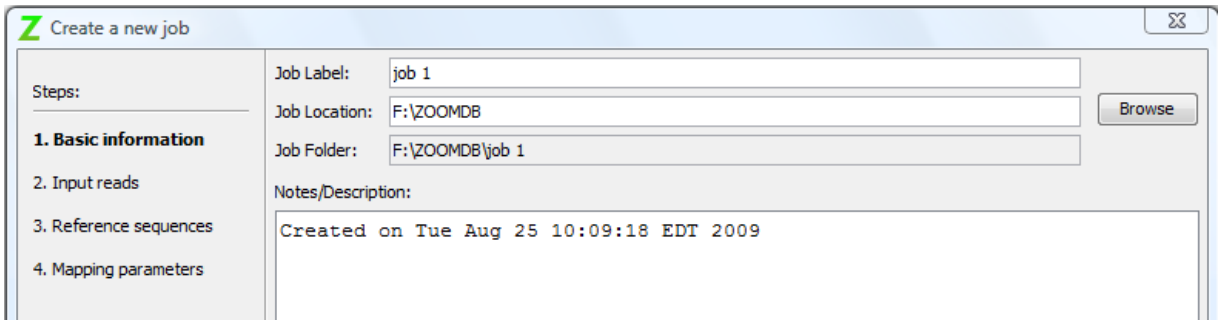
3.4 Create a Job

This will be a rather simple job as it will only contain one read file and one reference file, however, the same process can be used for jobs with reads directory or multiple reads files and multiple reference sequence files. Click on the "Create a new job" toolbar icon " " or select "New Job" from the "File" menu. The following window will appear:



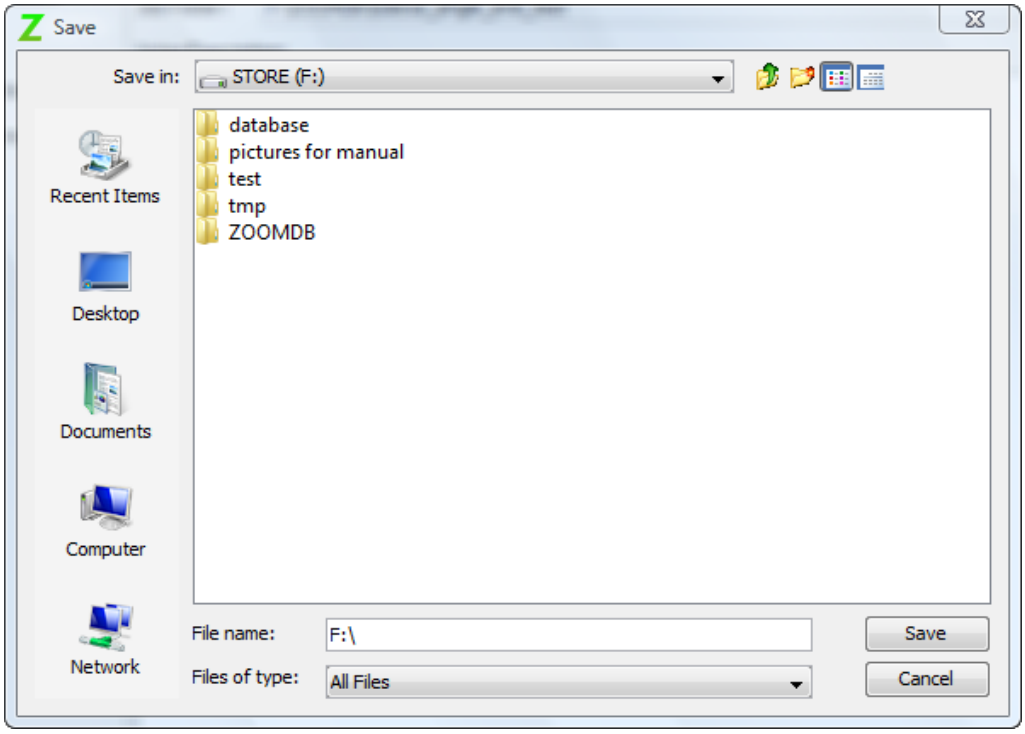
Basic information

This part is used to assign a name for your job and a directory to store the data related to your job. After you finished the job, you can load the job to display results or post-analysis.

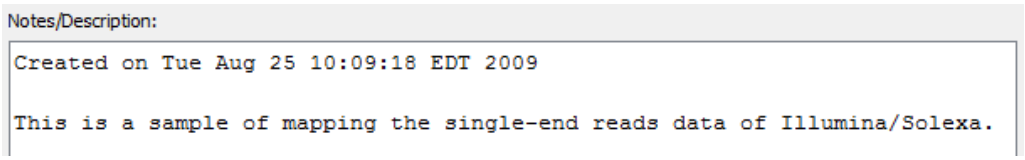


1. Enter a name for your job in the blank field beside the “Job Label”, for example “Solexa_single_end_test”.

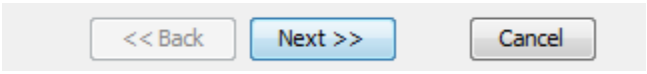
2. Press the "Browse" button to specify a directory to save your job. For example, "F:\ZOOMDB".



3. You can enter any descriptions about your job for later reference.


Notes/Description:
Created on Tue Aug 25 10:09:18 EDT 2009
This is a sample of mapping the single-end reads data of Illumina/Solexa.

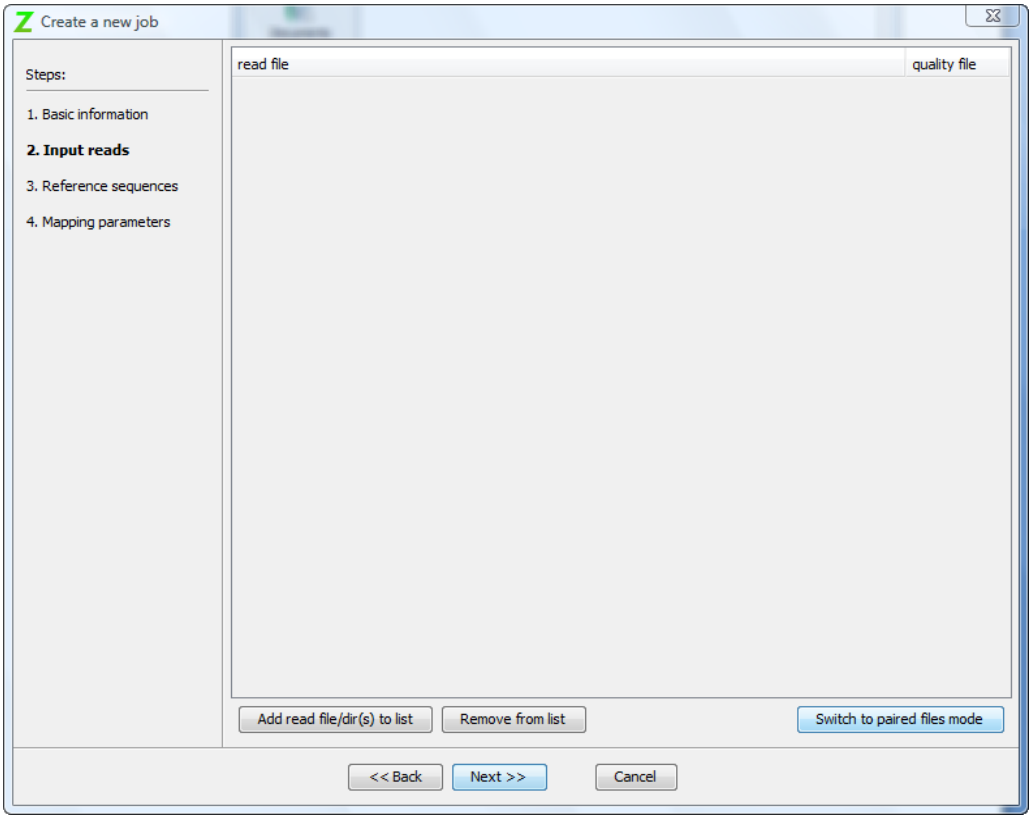
4. Click "Next" button on the bottom of the window to continue.



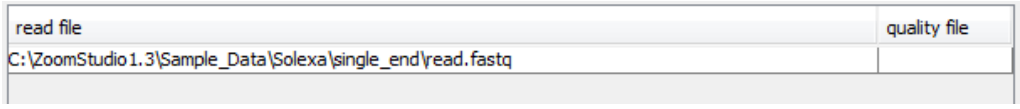
Input reads

All reads data are input here.

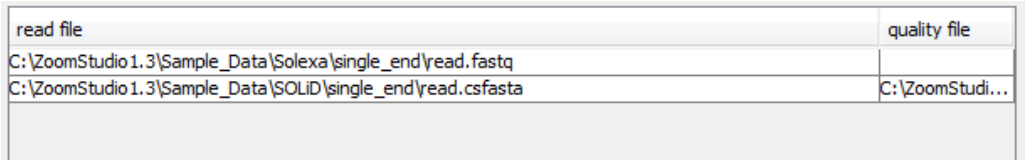
There are two ways to input reads files, by selecting read files or directories. ZOOM will automatically search for all the reads file inside. Please note that the read file should be in a standard format of next generation sequencing technologies. For example, "*_seq.txt", "*_qual.txt", "*.fastq", "*.fasta" files for Illumina data, or"*.csfasta", "*_QV.qual","*.fastq" files for ABI SOLiD data. For details, please refer to Chapter 4 in this manual.



1. Click the "Add read file/dir(s) to list" button, navigate to "Sample_Data\Solexa\single_end" directory and select the "read.fastq" file. The file will be selected in the read file list.

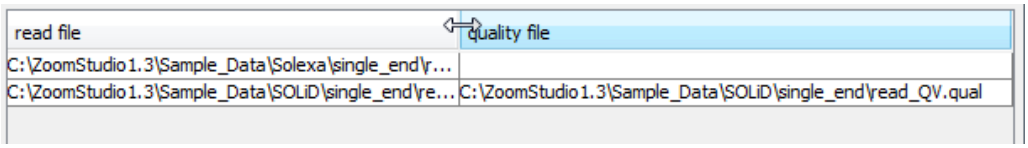


2. Click the "Add read file/dir(s) to list" button again to select other reads files. For example, select "read.csfasta" file in the "Sample_Data\SOLiD\single_end" directory. Then the "read.csfasta" will be loaded into the read file list too.



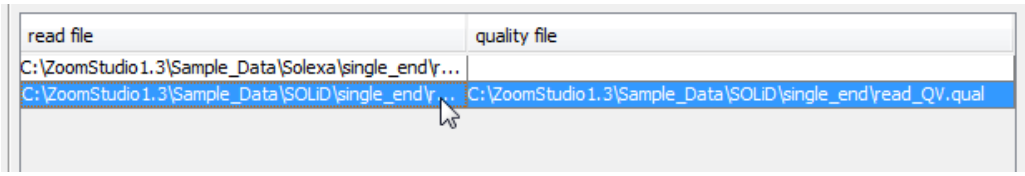
Note that ZOOM also recognizes that the "read.csfasta" file has a corresponding quality file "read_QV.qual". It will load the quality file too.

By clicking and dragging the mouse on the boundary between the "read file" and "quality file" headers, you can tune the width of the tablet and show the full name of the quality files, as follows:

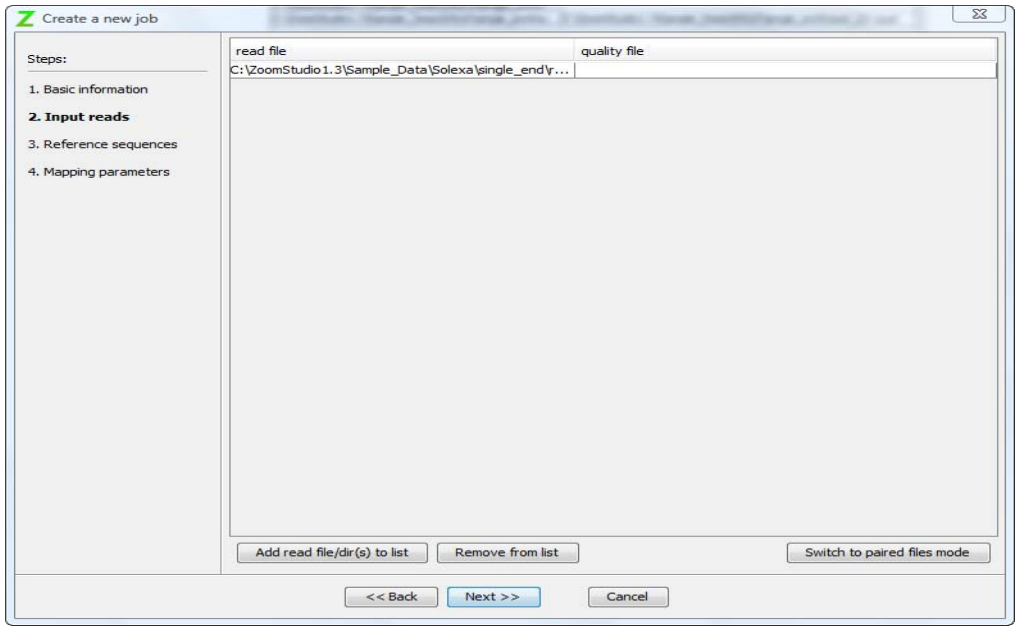


ZOOM recognizes the corresponding quality file by the file names so please make sure that the read sequence file is in the same directory with the quality score file and the prefixes of the file names are same. For Illumina/Solexa data, the "<filename>_seq.txt" will be matched with "<filename>_qual.txt". For ABI SOLiD data, the "<filename>.csfasta" will be matched with "<filename>_QV.qual". The quality score in the FASTQ format will be loaded directly.

3. By selecting the files you don't want and clicking "Remove from list", you can remove these files. Select the "read.csfasta" file in the read file list as following and click the "Remove from list" button.



This file will then be removed from the read file list.

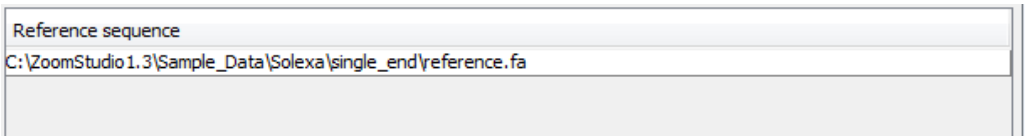


4. Click the "Next" button on the bottom of the window to continue.

Reference sequences

Assign the reference sequences where the reads data are mapped to.

1. Press the "Add file/dir(s) to list" button, and choose the reference sequence "reference.fa" in the "Sample_Data\Solexa\single_end" directory.

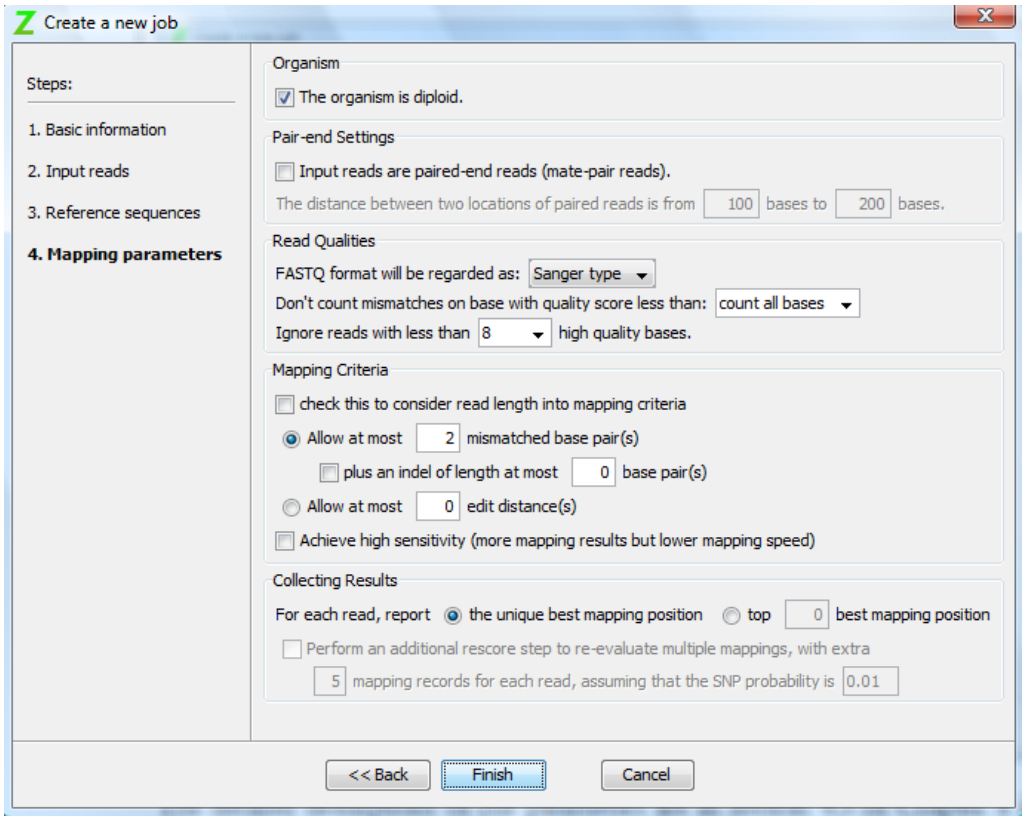


The sequences in the reference files should be in FASTA format. Multiple reference files or a directory can be loaded in. Use the "Remove from list" button to remove files if needed.

2. Click the "Next" button on the bottom of the window to continue.

Mapping parameters

Please use the following default parameters:


Create a new job
Steps:
1. Basic information
2. Input reads
3. Reference sequences
4. Mapping parameters
Organism
The organism is diploid.
Pair-end Settings
Input reads are paired-end reads (mate-pair reads).
The distance between two locations of paired reads is from 100 bases to 200 bases.
Read Qualities
FASTQ format will be regarded as: Sanger type
Don't count mismatches on base with quality score less than: count all bases
Ignore reads with less than 8 high quality bases.
Mapping Criteria
check this to consider read length into mapping criteria
Allow at most 2 mismatched base pair(s)
plus an indel of length at most 0 base pair(s)
Allow at most 0 edit distance(s)
Achieve high sensitivity (more mapping results but lower mapping speed)
Collecting Results
For each read, report the unique best mapping position top 0 best mapping position
Perform an additional rescore step to re-evaluate multiple mappings, with extra
5 mapping records for each read, assuming that the SNP probability is 0.01
<< Back
Finish
Cancel

The detailed descriptions of the parameters are in Section 4.3 of Chapter 4 in this manual.

Click the " " button. A new job will be created. A directory named "Sample_single_end_test" will be created. All information about this job will be stored in this directory. You can copy this directory anywhere. If you use ZOOM to load in this directory, the job can be shown and post-analysis can be carried out on it.

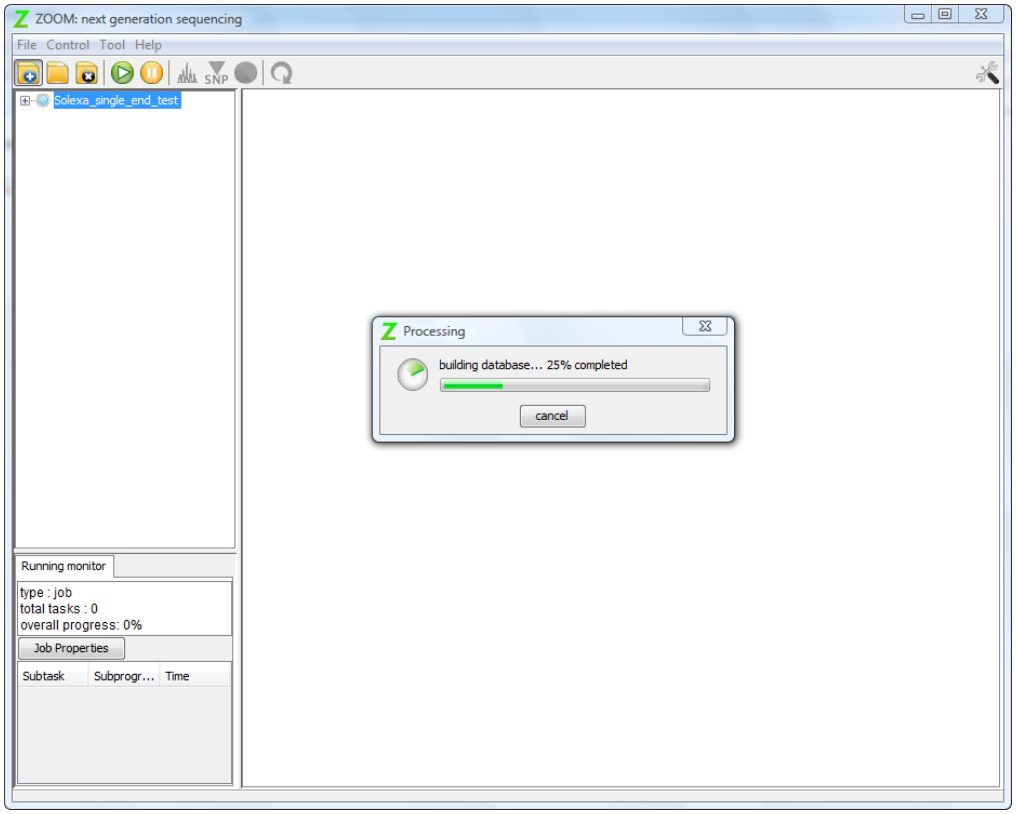
3.5 Monitor the job

After the job is created, the job will be shown in the "Job View" panel in the left window of the interface. For each job, ZOOM will automatically create a "task" to map these reads on the assigned server. If the amount of reads is large, ZOOM will automatically partition the reads into several parts and launch several tasks for each part of the reads. ZOOM will schedule these tasks automatically until all reads are handled, and the user can monitor the running status of the

jobs and the tasks according to the corresponding progress bars in the “Running Monitor” window.

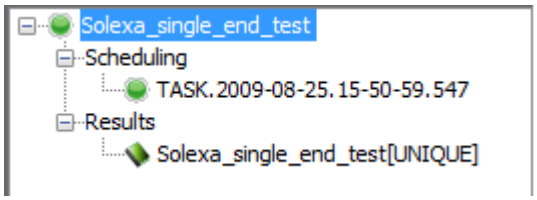
Progress Bar

Depending on the data size, it may take some time to load the data. The time is related to the data size of the reads data file. A progress bar will pop up showing the progress of loading data. ZOOM won’t respond until the progress bar has disappeared.



Job View panel

After loading the data, you will see the job in the “Job View” panel:



The “Job View” panel which is shown in the upper left hand corner displays the organization of a particular job. Use the ‘+’ and ‘-’ boxes to expand and collapse the job in order to know the organization of this job. In each job node, there is a

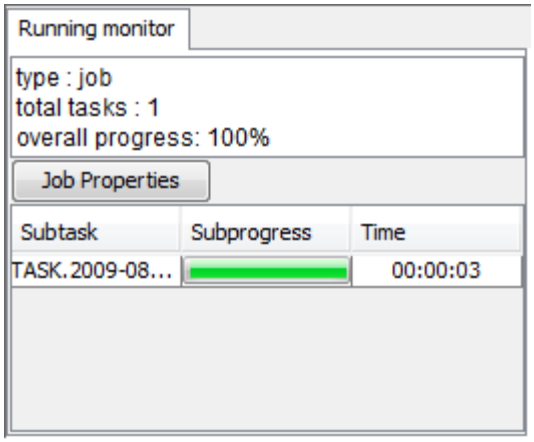
"Scheduling" node and a "Results" node.

The "Scheduling" node shows all the tasks this job has been split to and scheduled on the server.

The "Results" node will not appear until all reads mapping tasks are finished. It will contain the uniquely mapped results (suffixed by "[UNIQUE]") and the top N mapping results (suffixed by "[ALL]") according to the running parameters.

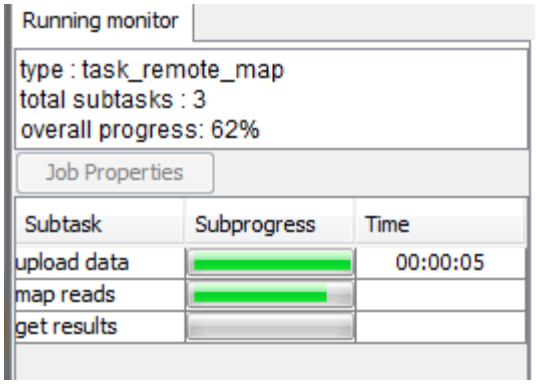
Running status of the job

Clicking on the job node, the "Running Monitor" will show the progress of the job.



Click the "Job Properties" button to display the properties of this job, including the read files and the reference files, using parameters and mnemonic notes.

Click on a task node. The progress of the task will be shown.



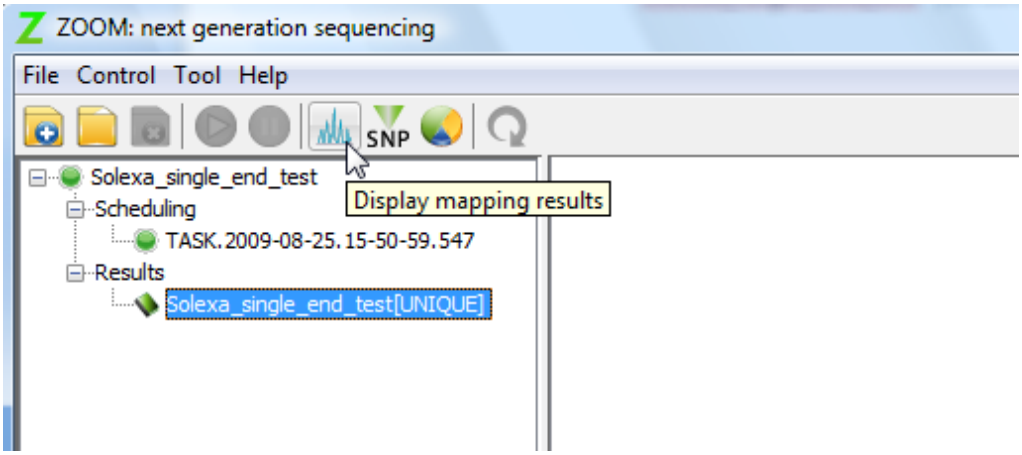
Control the job

If you want to cancel or restart a job or several jobs, choose the corresponding job nodes, and then click the " " tool bar icon or the " " toolbar icon.

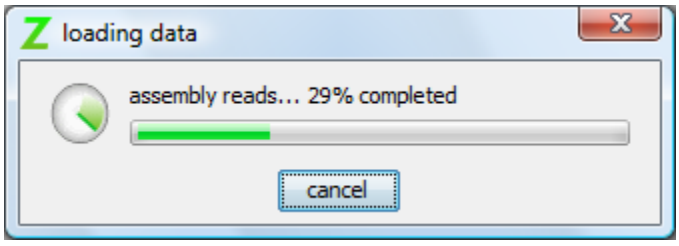
3.6 Display Mapping Results

When the job icon turns into "", the job is finished. You can show mapping results or carry out SNP analysis now. Make sure that you select the node under the "Results" node when choosing data to be analyzed.

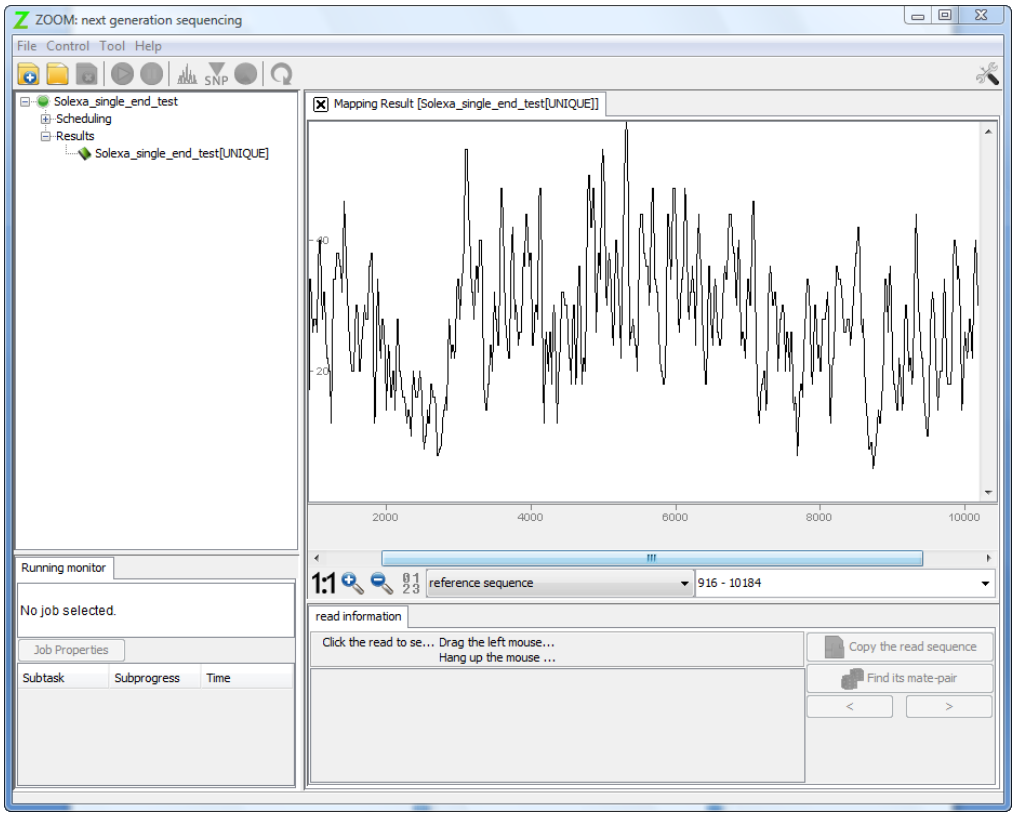
1. Select the "UNIQUE" node in the "Results" node on the "Job View" panel and click the "Display mapping result" toolbar icon "".



2. ZOOM will assemble the mapped reads into a consensus sequence and show the read depth overview along the reference sequence. This will take some time depending on the amount of mapped reads and the length of the reference sequence. A progress bar will pop up.



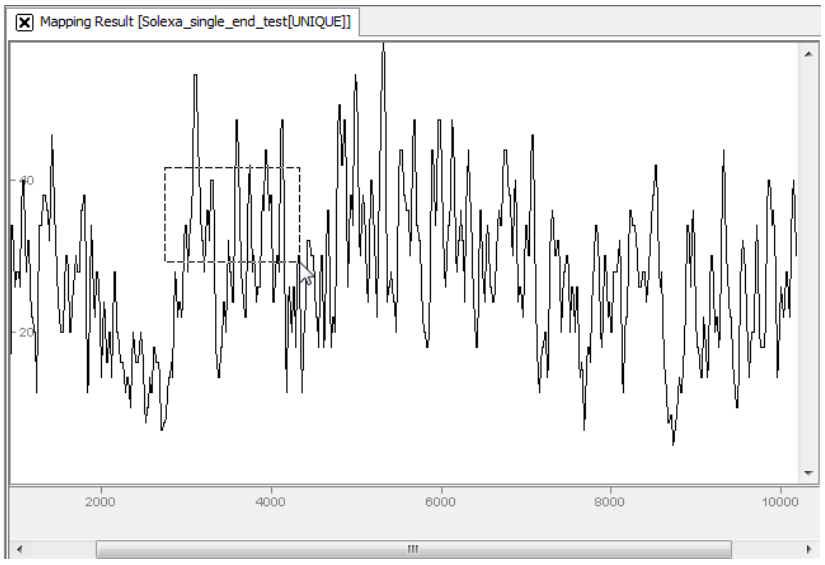
3. After the progress is finished, you can see a tabbed window containing the mapping results on the right hand of the main window of ZOOM as follows:



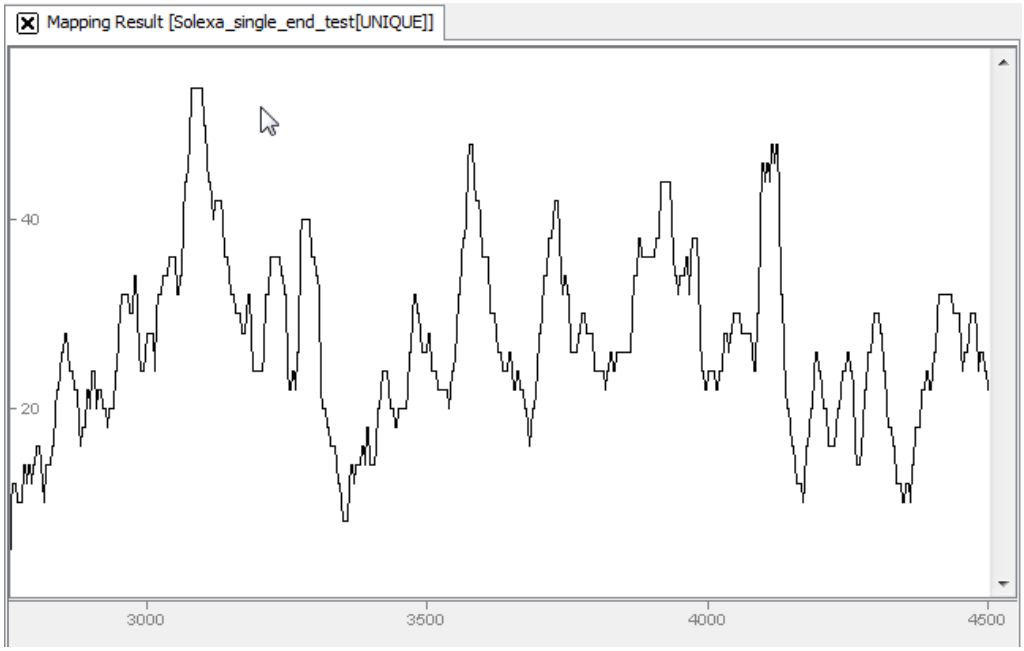
The line in the graph is the overview of the read depth of those mapped reads along the reference sequence. The horizontal ruler denotes the positions on the reference sequence. The vertical ruler denotes the read depth.

4. Press " " button to zoom in the graph or press " " to zoom out in the graph.

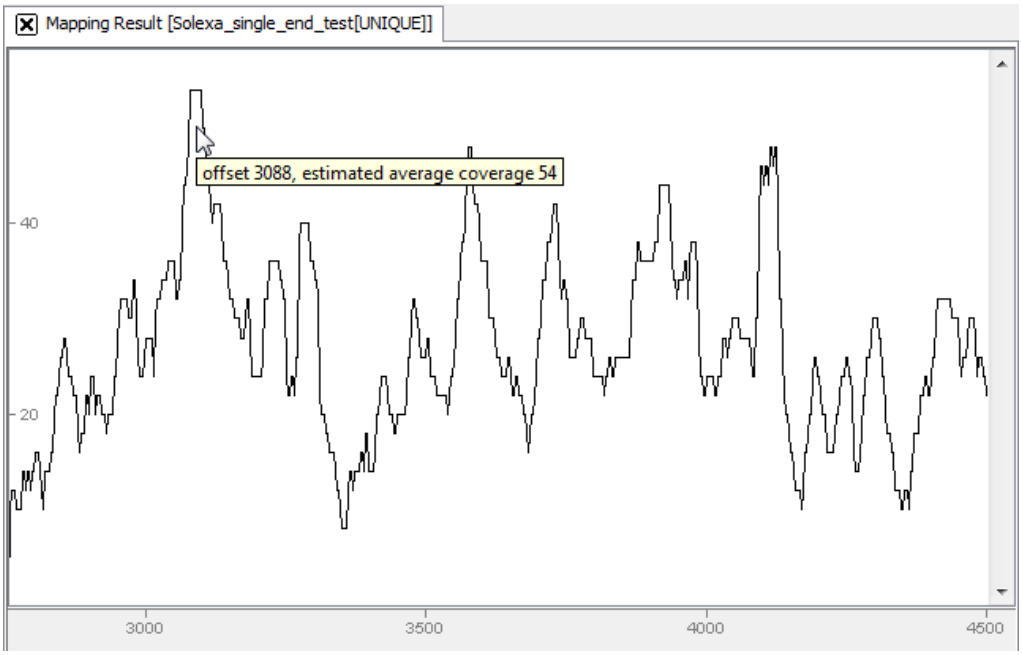
5. Click the left button on your mouse and drag along the graph to form a rectangle region, and then release the mouse button.



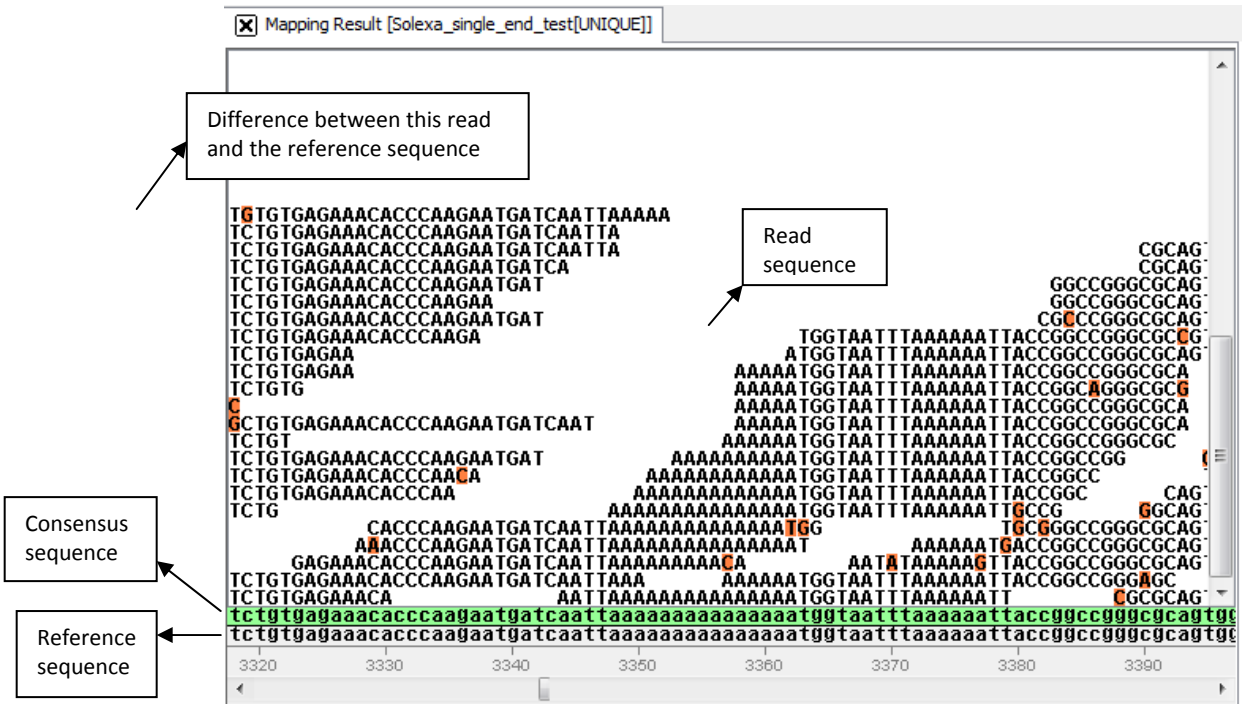
The selected rectangle region will be enlarged to the full window of the “Mapping Results Displaying Window” as follows:



6. Rest the cursor on a position of the peaks for a second. The average read depth of this position will be shown in a tooltip box besides the mouse.



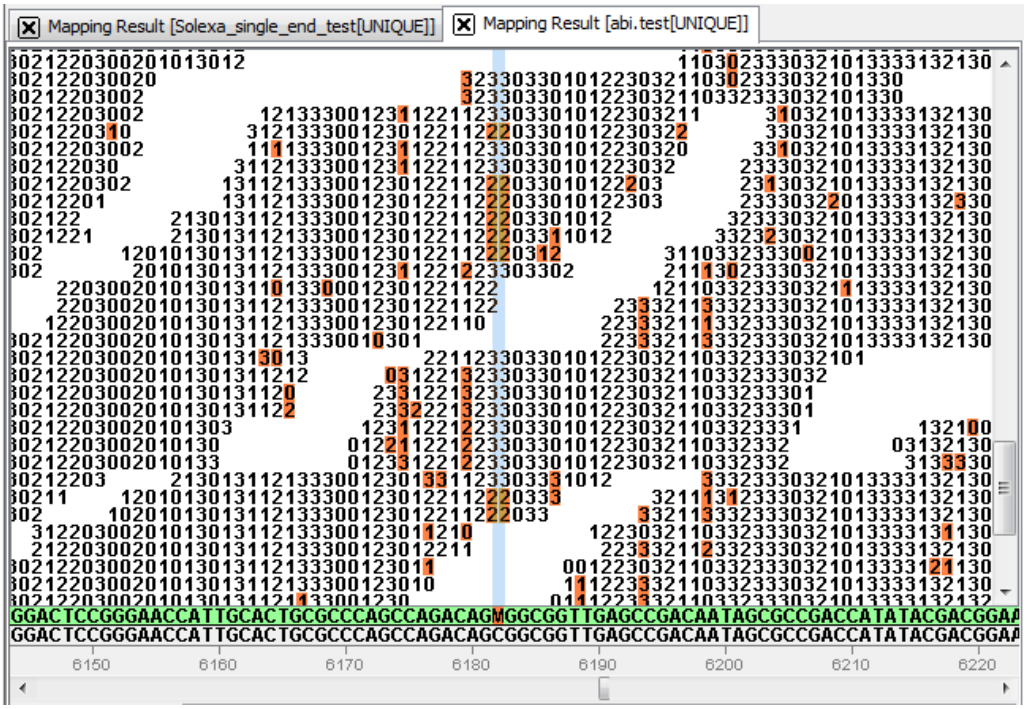
7. Click on a place in the “Mapping Results Displaying window”. The detailed alignments of the mapped reads along the reference sequence will be shown as follows:



The sequence at the bottom of the window is the reference sequence. The sequence with green background over the reference sequence is the consensus sequence generated by the mapped reads along the reference sequence.

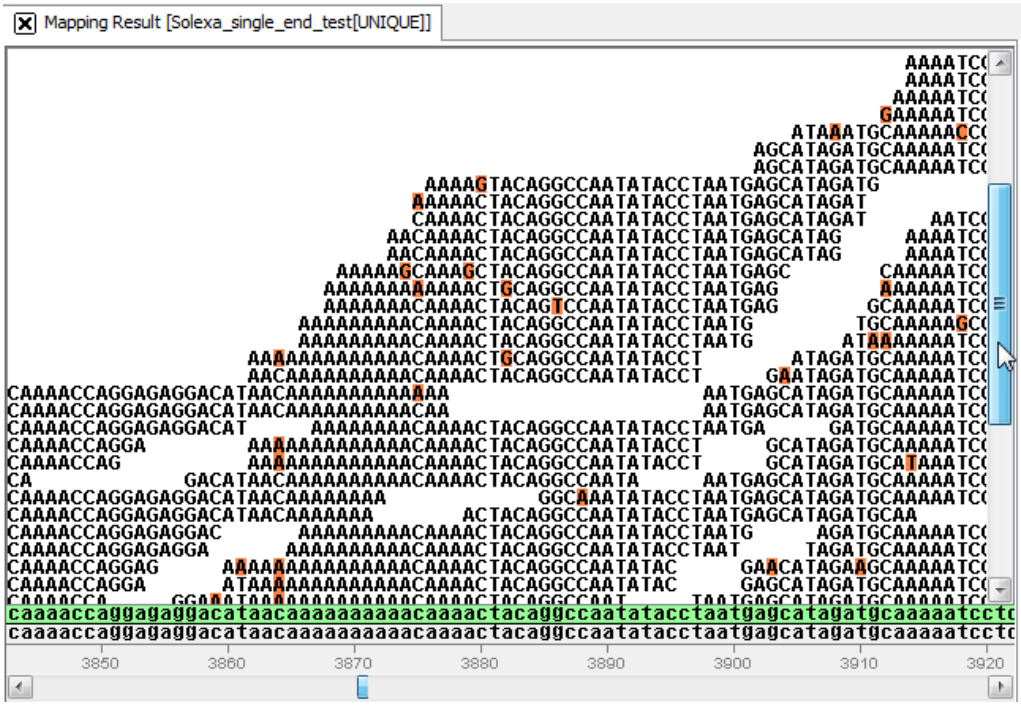
The orange background of the nucleotides on the read or the consensus sequence highlights the difference from the nucleotide on the position of the reference sequence.

The default display of the read is in the nucleotide space. For ABI SOLiD data, the default display is the decoded nucleotide reads according to the mapping results. Press “ ” button to switch the reads display from the nucleotide space to the color space, and “ ” vice versa. The reads shown in color space look like the following:



8. Click or drag the horizontal scrollbar will let you navigate along the reference sequence.

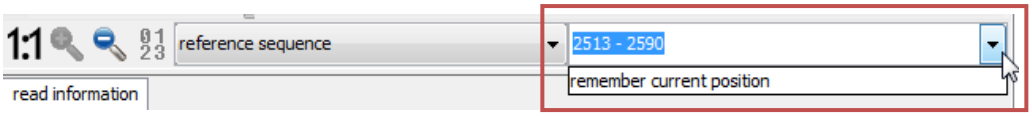
9. Click or drag the vertical scrollbar on the right to show more reads aligned to this region when all the reads mapped here cannot fit in the “Mapping Results Window”.



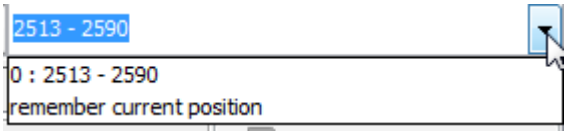
Click on the “Reference Sequence Selecting Bar”. The reference sequence name list will be displayed. If there are multiple reference sequences, there will be a dropdown list where you can choose one reference sequence to show the alignments on it.

In this case, there is only one reference sequence named “reference sequence”.

10. Click on the “Locating bar”.



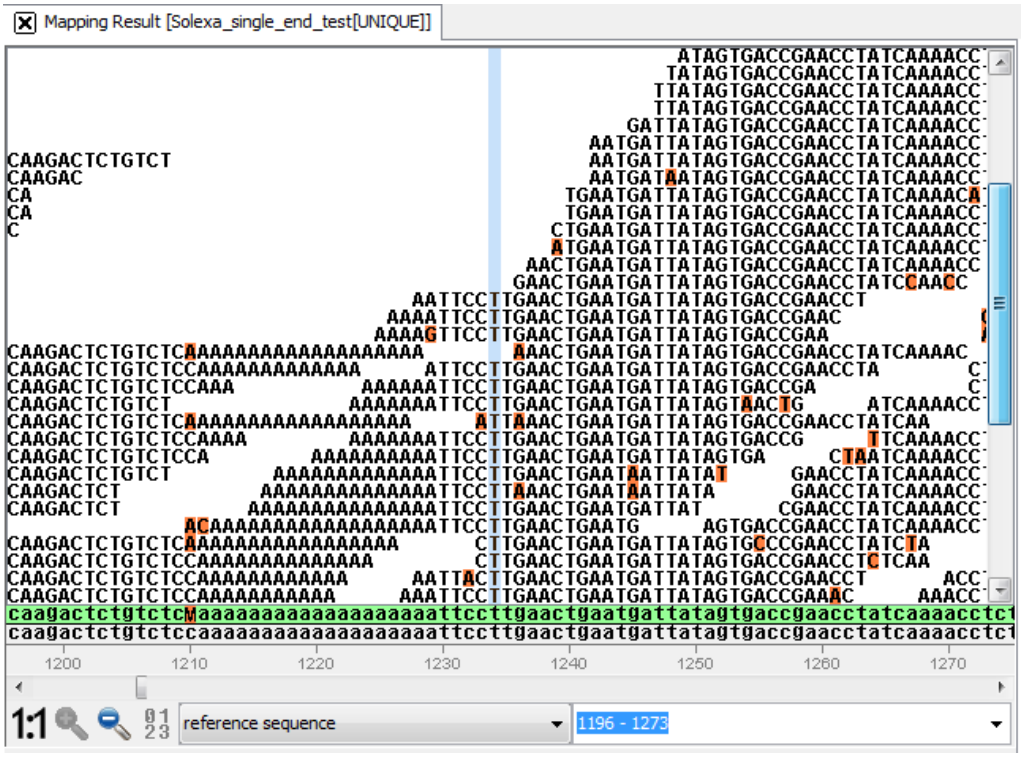
The “2513-2500” (you may see different numbers) is the offsets of current showing range in the “Mapping Results Illustrating window” on the reference sequence. Click on “remember current position”, and click the “Locating bar” again. You will see:



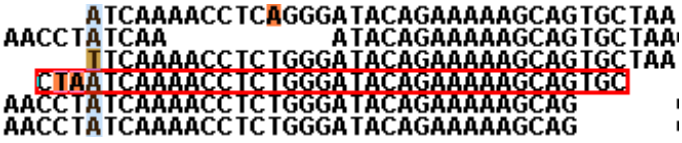
"0:2513-2590" is recorded here, and by selecting this entry, you can go back to this region at any time.

Enter a new position or a position range in the "Locating bar" such as "1234" or "1234-4560". Then read alignments in the new region will be shown in the "Mapping Results Illustrating window".

11. Enter a single position such as "1234" in the "Locating bar" or click on a column in the ""Mapping Results Illustrating window". A light blue bar will highlight this position as follows:

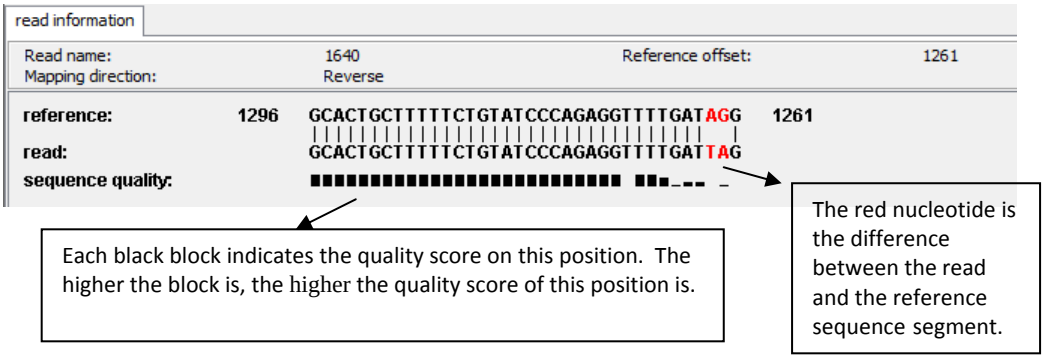


12. Click on any read in the "Mapping Results Illustrating window".



The read will be highlight by a red rectangle.

At the same time, more information of this mapped read will be shown in the "read information" tab window below the "Mapping Results Illustrating window":

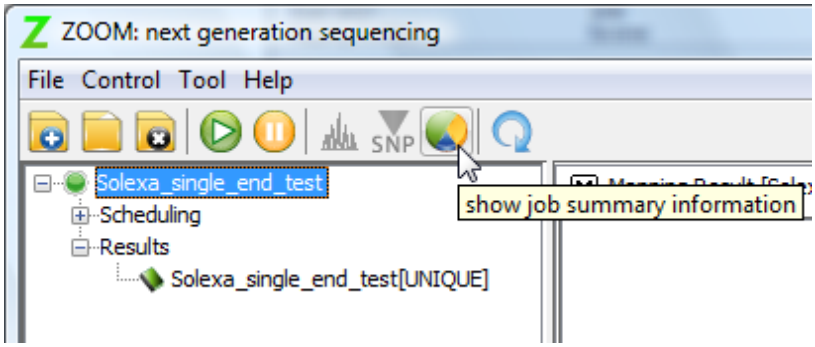

read information
Read name: 1640
Mapping direction: Reverse
Reference offset: 1261
reference: 1296 GCACTGCTTTTTCTGTATCCCAGAGGTTTTGATAGG 1261
read: GCACTGCTTTTTCTGTATCCCAGAGGTTTTGATTAG
sequence quality:
Each black block indicates the quality score on this position. The higher the block is, the higher the quality score of this position is.
The red nucleotide is the difference between the read and the reference sequence segment.

Note that the direction of the alignment shown in the "read information" tab is the same as the direction of the read sequence in the read files. If a read is mapped to the reverse chain of the reference sequence, the reference segment is reversed and the left offset is larger than the right offset as shown in the above picture.

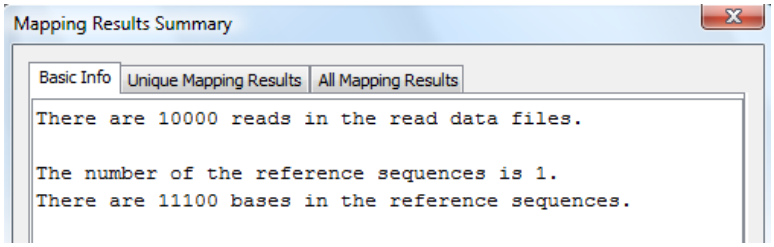
13. Click the "

" button, then the read name and the read sequence will be copied to the clipboard of system.

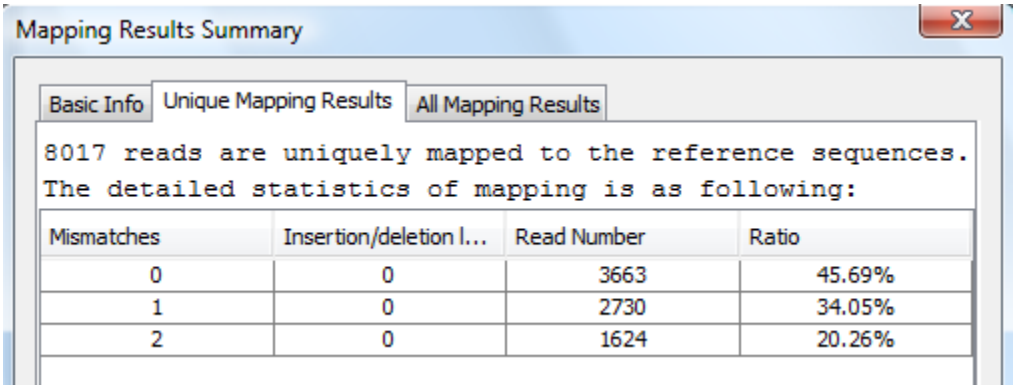
14. Click the "Solexa_single_end_test" job node and click " " toolbar icon.



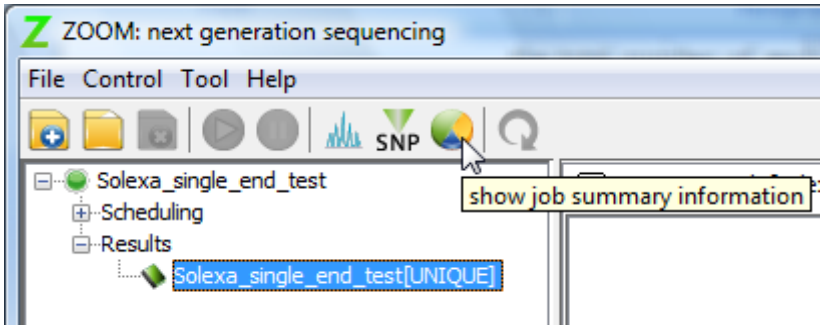
A summary of the mapping results will be shown in the pop up window. The summary includes the total number of reads in the read data files, the number of reference sequences and the length of the reference sequences:


Mapping Results Summary
Basic Info
Unique Mapping Results
All Mapping Results
There are 10000 reads in the read data files.
The number of the reference sequences is 1.
There are 11100 bases in the reference sequences.

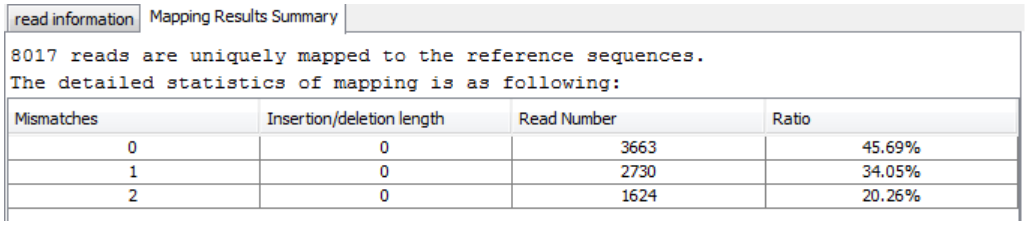
Click on the “Unique Mapping Results” tab to show the number of reads mapped uniquely and the statistics of the uniquely mapping results:



15. Click the “UNIQUE” results node, and click the “ ” toolbar icon.



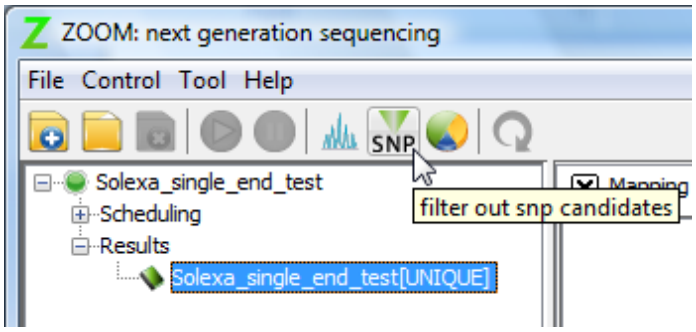
The summary of the uniquely mapped results will be show in a “Mapping Summary” tab window beside the “read information” tab.



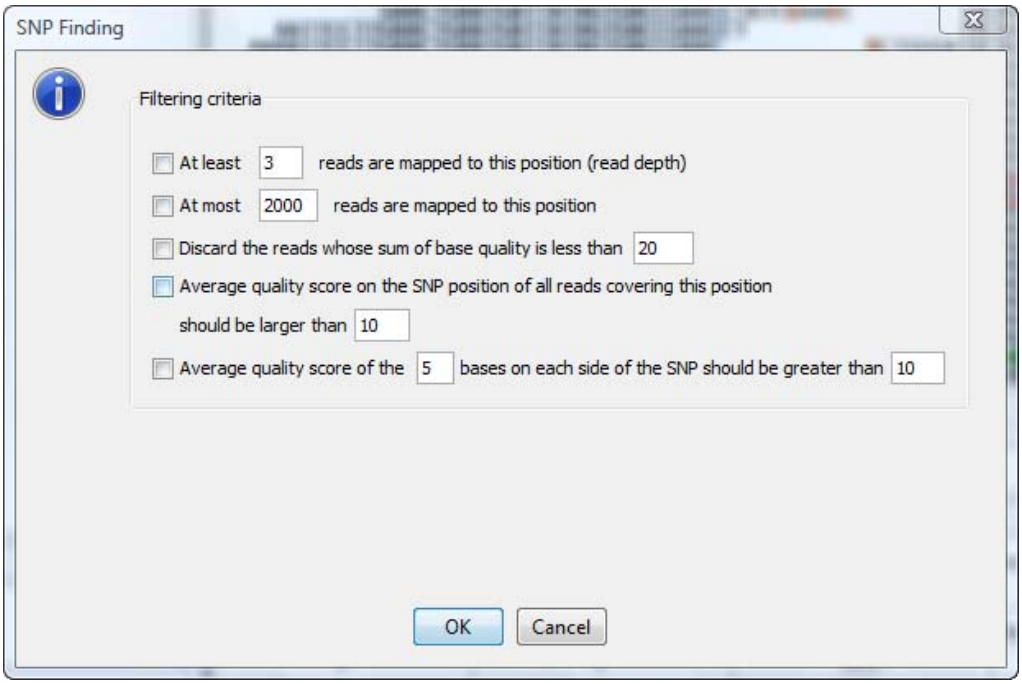
3.7 Finding SNP Candidates

We suggest that users find SNP candidates only using the uniquely mapped reads (i.e. using the [UNIQUE] result node other than [ALL] result node). Because the [All] result node contains top N mapping results for each read, those reads mapped to multiple positions of the reference sequence will make the SNP finding process unreliable.

1. Click the "Solexa_single_end_test[UNIQUE]" result node, and click the "filter SNP candidates" toolbar icon "SNP" (or Select "SNP Filter" from the "Tools" menu).

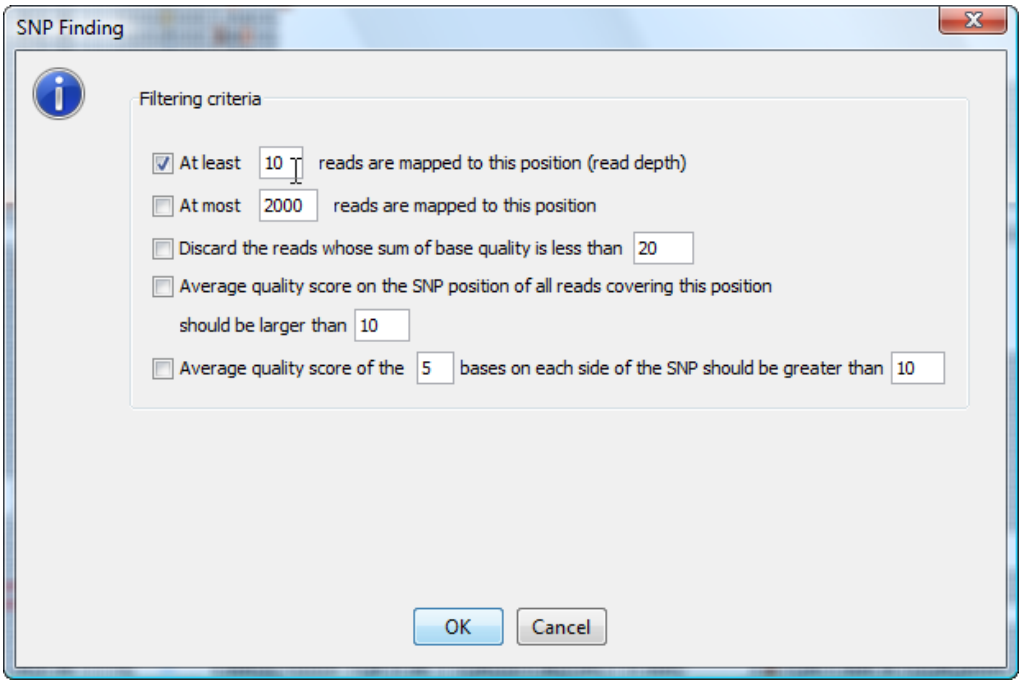


A window showing "Filter criteria" will pop up as follows:

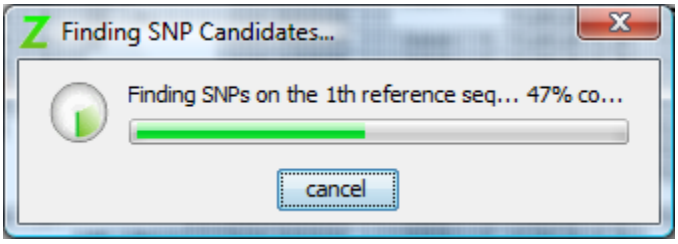

SNP Finding
Filtering criteria
At least 3 reads are mapped to this position (read depth)
At most 2000 reads are mapped to this position
Discard the reads whose sum of base quality is less than 20
Average quality score on the SNP position of all reads covering this position
should be larger than 10
Average quality score of the 5 bases on each side of the SNP should be greater than 10
OK
Cancel

There are five filtering criteria which you can apply for the SNP finding. For a detailed explanation of each criterion, please refer to Section 6.1 in the Chapter 6 in this manual.

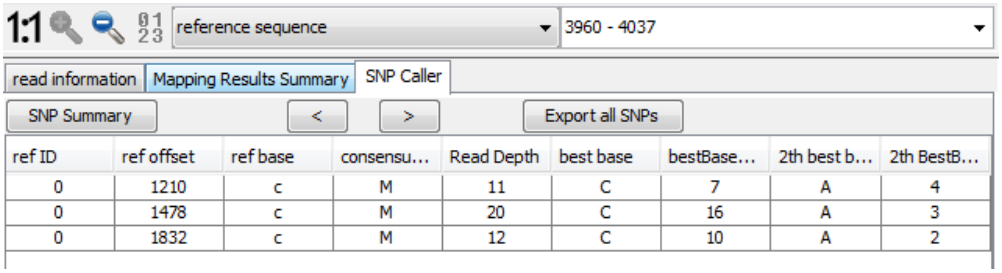
2. Click on the checkbox of the filtering criterion “At least … reads are mapped to this position” and revise the value to 10.


SNP Finding
Filtering criteria
At least 10 reads are mapped to this position (read depth)
At most 2000 reads are mapped to this position
Discard the reads whose sum of base quality is less than 20
Average quality score on the SNP position of all reads covering this position
should be larger than 10
Average quality score of the 5 bases on each side of the SNP should be greater than 10
OK
Cancel

3. Press “OK” button. Then SNP finding on all the reference sequences will be carried out. A progress bar will pop up:



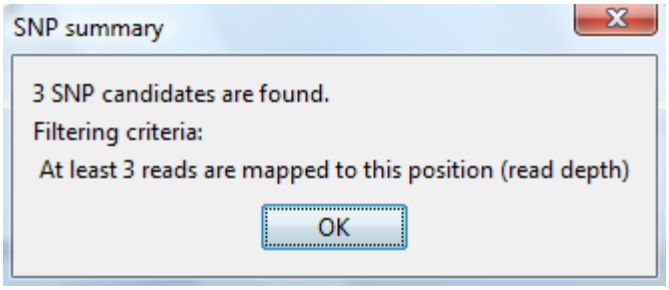
4. When all SNP candidates are located, a table containing SNP candidates will appear in the “SNP Caller” tab as follows:



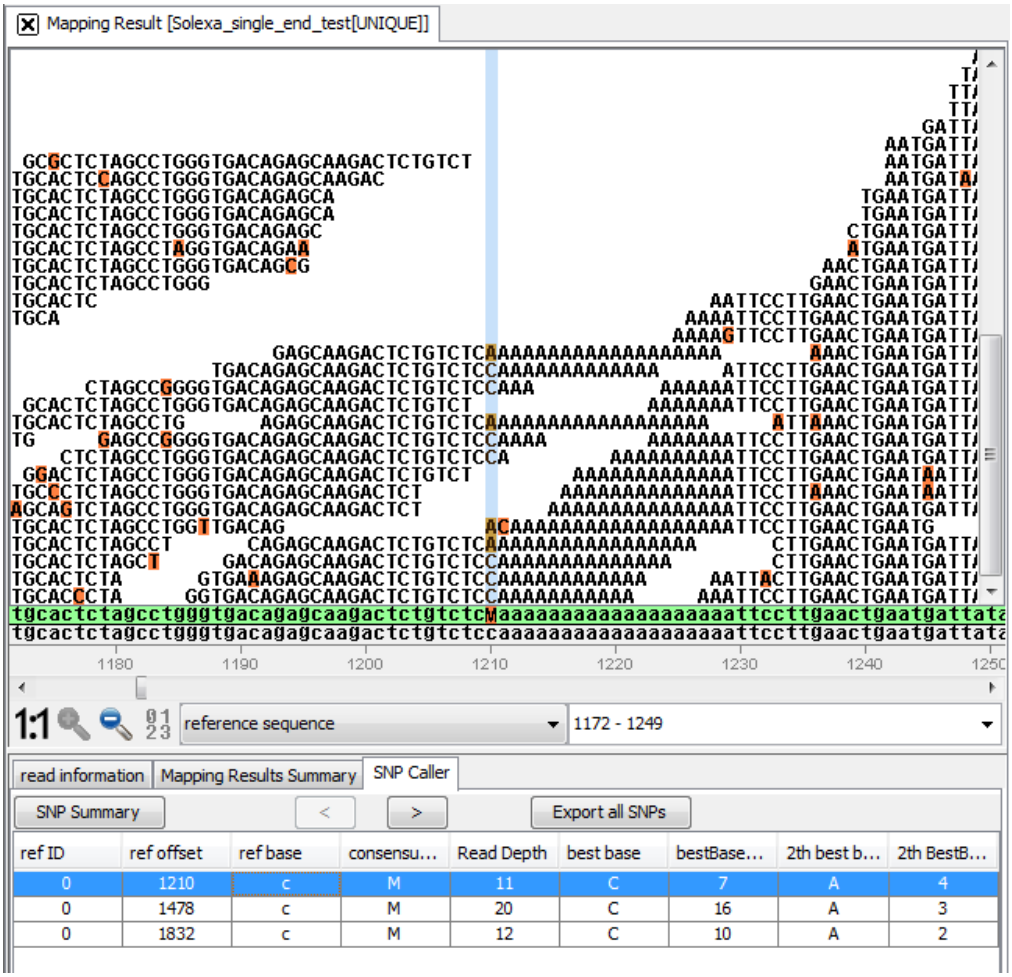
ref ID	ref offset	ref base	consensu...	Read Depth	best base	bestBase...	2th best b...	2th BestB...
0	1210	c	M	11	C	7	A	4
0	1478	c	M	20	C	16	A	3
0	1832	c	M	12	C	10	A	2

Each row of the table is a SNP candidate. The table has 9 fields showing 9 features of each SNP. The description of each field is in Section 6.2 in Chapter 6.

Click the "SNP Summary" button. The amount of SNP candidates satisfying the filtering criteria and the filtering criteria adopted will be shown:

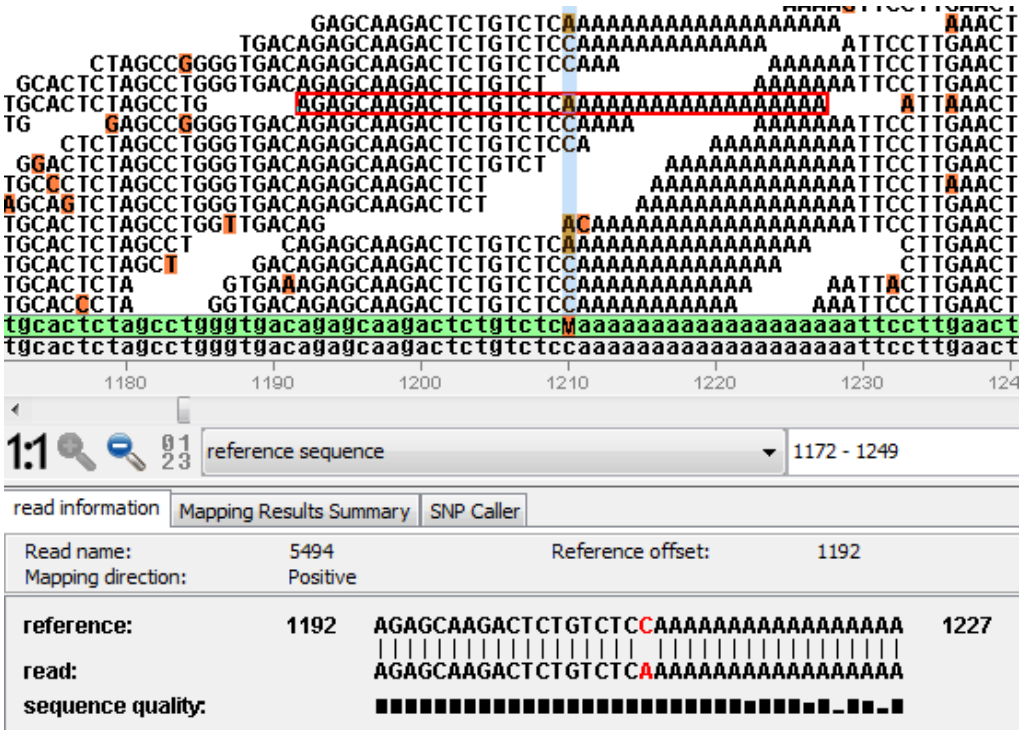


5. Double click the first row in the table to show the first SNP.


Mapping Result [Solexa_single_end_test[UNIQUE]]
1180
1190
1200
1210
1220
1230
1240
reference sequence
1172 - 1249
read information
Mapping Results Summary
SNP Caller
SNP Summary
Export all SNPs
ref ID | ref offset | ref base | consensu... | Read Depth | best base | bestBase... | 2th best b... | 2th BestB...
0 | 1210 | c | M | 11 | C | 7 | A | 4
0 | 1478 | c | M | 20 | C | 16 | A | 3
0 | 1832 | c | M | 12 | C | 10 | A | 2

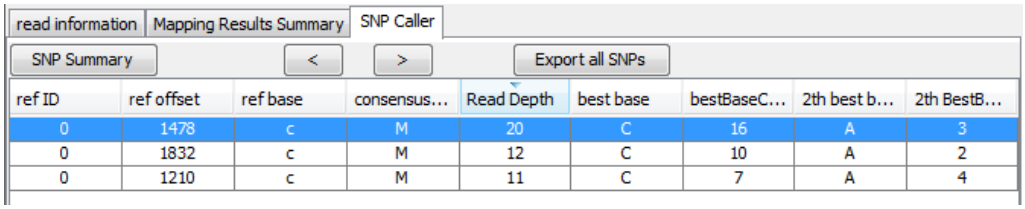
The light blue bar will highlight the SNP position. You can check the alignment around this position in detail. You can double click each row in the table to see the SNP candidate details.

6. Click one read in this position and click the “read information” tab. You can check the quality of the position of this read to know whether the SNP candidate is more likely a true SNP or a sequencing error.



7. Click the “SNP Caller” tab to show the SNPs (show what??). Click the “ < ” or the “ > ” button to jump to the previous or the next SNP candidate.

8. Click the “Read Depth” field in the header of the SNP table to sort the candidates according to the read depth in ascending order. Click it again to sort in descending order. Similarly each field in the SNP table can be sorted.



ref ID	ref offset	ref base	consensus...	Read Depth	best base	bestBaseC...	2th best b...	2th BestB...
0	1478	c	M	20	C	16	A	3
0	1832	c	M	12	C	10	A	2
0	1210	c	M	11	C	7	A	4

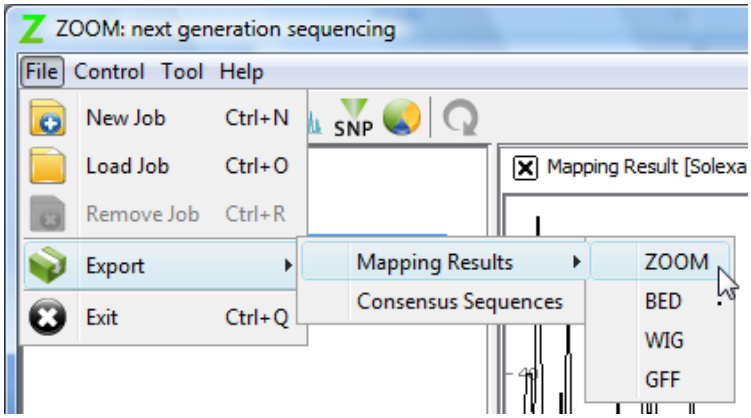
9. Click the “ Export all SNPs ” button to export the SNP candidates into a file. All SNP candidates will be exported in a format of the nine fields as each line in the SNP table.

3.8 Export data into files

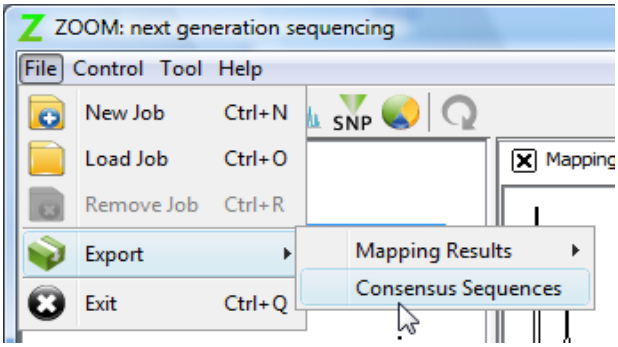
The mapping results and consensus sequence can be exported to files. Note that only results nodes can be exported.

1. Select the “Solexa_single_test[UNIQUE]” result node.
2. Select “Export” from the “File” menu. Select “Mapping Results” from the popup menu.

There are four output formats to export mapping results into. Please refer to Section 7.1 in Chapter 7 in this manual for the description of each format.



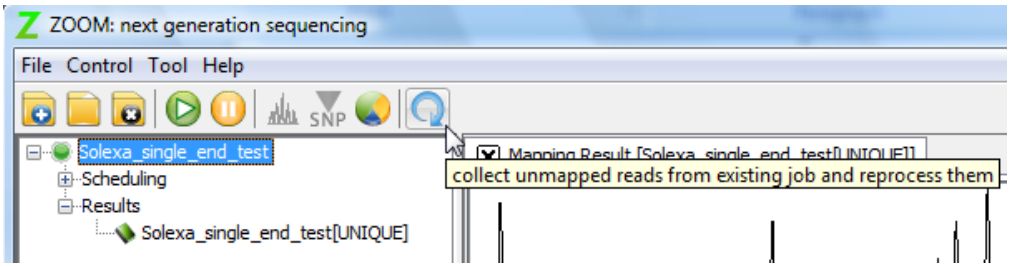
3. Select “Export” from the “File” menu. Select “Consensus Sequences” from the popup menu. The consensus sequence built according to the mapping results will be exported in FASTA format. Note that we suggest only building a consensus sequence on the [UNIQUE] result node based on similar reason for SNP finding.



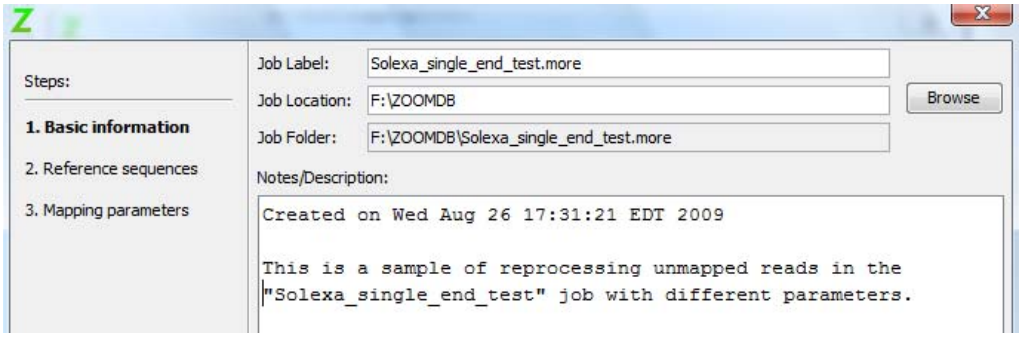
3.9 Change parameters to get more mapping results

For the unmapped reads of this job, adjusting parameters such as the reference sequences, mismatch number allowed between reads and reference sequences may achieve more mapping results.

1. Click the “Solexa_single_end” job node and click the “reprocess unmapped reads” toolbar icon “ ”.



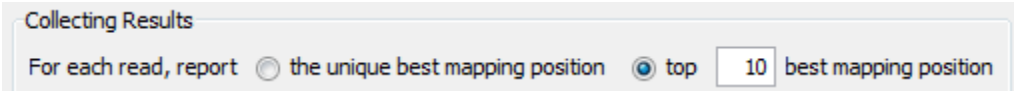
The following windows will pop up:


Steps:
1. Basic information
2. Reference sequences
3. Mapping parameters
Job Label: Solexa_single_end_test.more
Job Location: F:\ZOOMDB
Browse
Job Folder: F:\ZOOMDB\Solexa_single_end_test.more
Notes/Description:
Created on Wed Aug 26 17:31:21 EDT 2009
This is a sample of reprocessing unmapped reads in the "Solexa_single_end_test" job with different parameters.

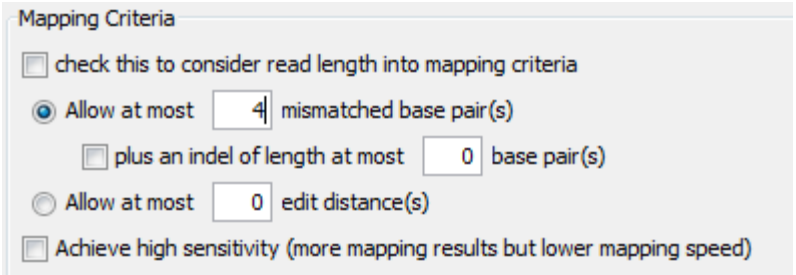
The process is similar to creating a new job, except that the reads data is the unmapped reads of the selected job. Assign a name to the new job for these unmapped reads. The default name is the original name suffixed by ".more".

2. Click "Next" twice to the "mapping parameters" step.

3. Check the radio box from the "the unique …" to "top…", and modify the value to 2, to keep up to 2 mapping results for each read.



4. Modify the mismatch number from 2 to 4, which will allow up to four mismatches between the reads and the reference sequences.

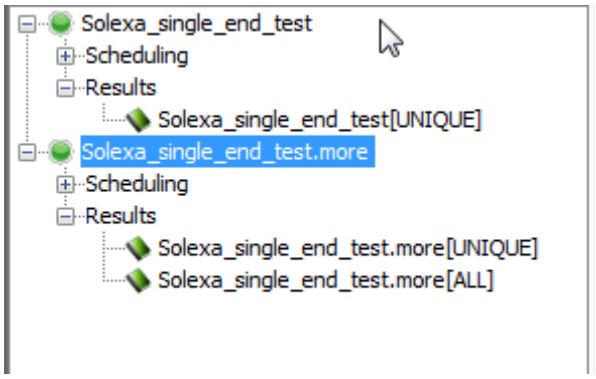


5. Click the check box to "achieve high sensitivity".

This will achieve full sensitivity to find all the mapping results with up to 4 mismatches. For more information on using this parameter, please refer to step 4 in Section 4.3 in Chapter 4.

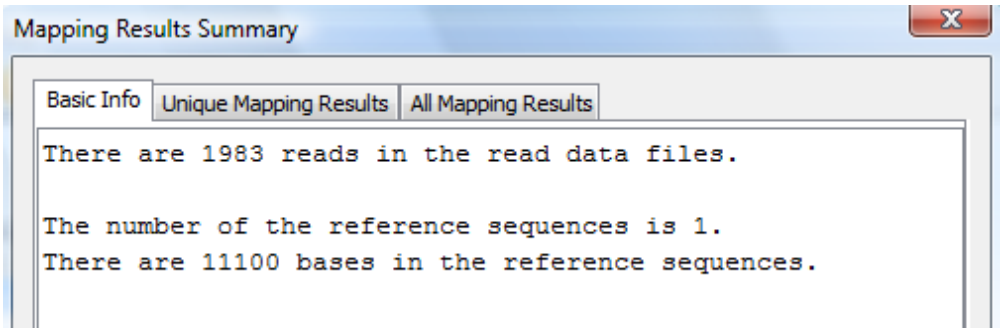
6. Click the "

" button to create this new job. A new job "Solexa_single_end_test.more" will be created and processed. After the new job is finished, there will be an additional job appearing in the "Job View panel" as follows:



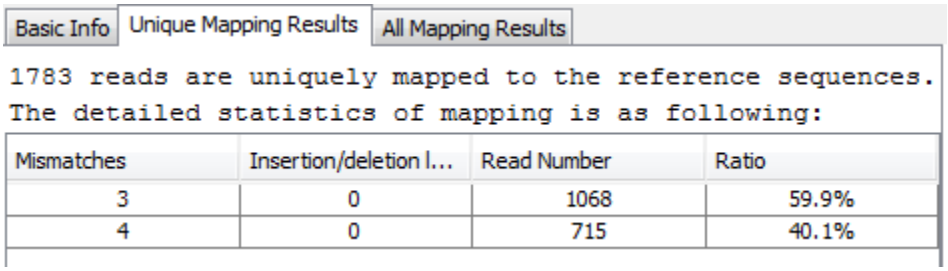
The new job has two Results nodes --- the [UNIQUE] and the [ALL] node because we set the parameters to collect top two mapping results for each read. The uniquely mapped result is in [UNIQUE] result node, while the top two mapping results are in the [ALL] node.

Click the " " toolbar icon. The job summary window will appear:

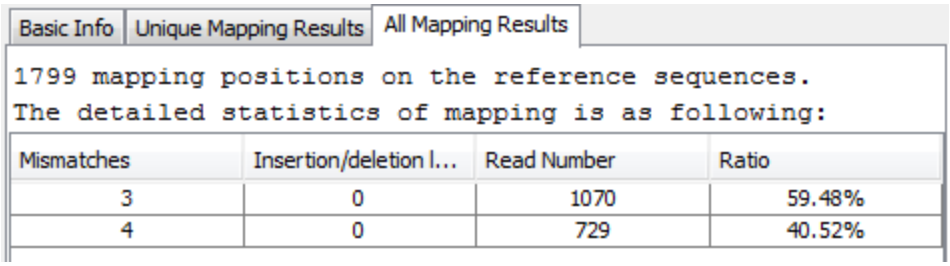

Mapping Results Summary
Basic Info
Unique Mapping Results
All Mapping Results
There are 1983 reads in the read data files.
The number of the reference sequences is 1.
There are 11100 bases in the reference sequences.

1983 reads are unmapped in the "Solexa_single_end_test" job. There are two summaries for uniquely mapped results and the top two mapped results, respectively.

7. Click the "Unique Mapping Results" tab. You can see that 1783 reads are mapped after increasing the mismatch number from 2 to 4 between the reads and the reference sequence.



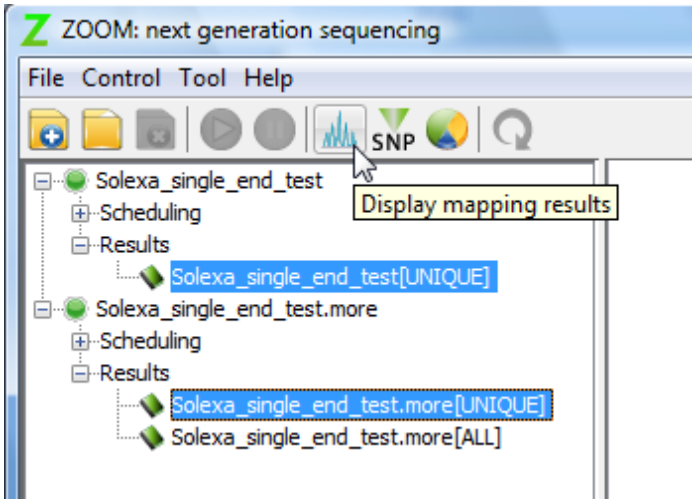
8. Click the "All Mapping Results" tab. There are 1795 mapping positions in the top two mapping results. Note that this is the number of mapping positions rather than the number of mapped reads, because one read might be mapped to multiple positions.



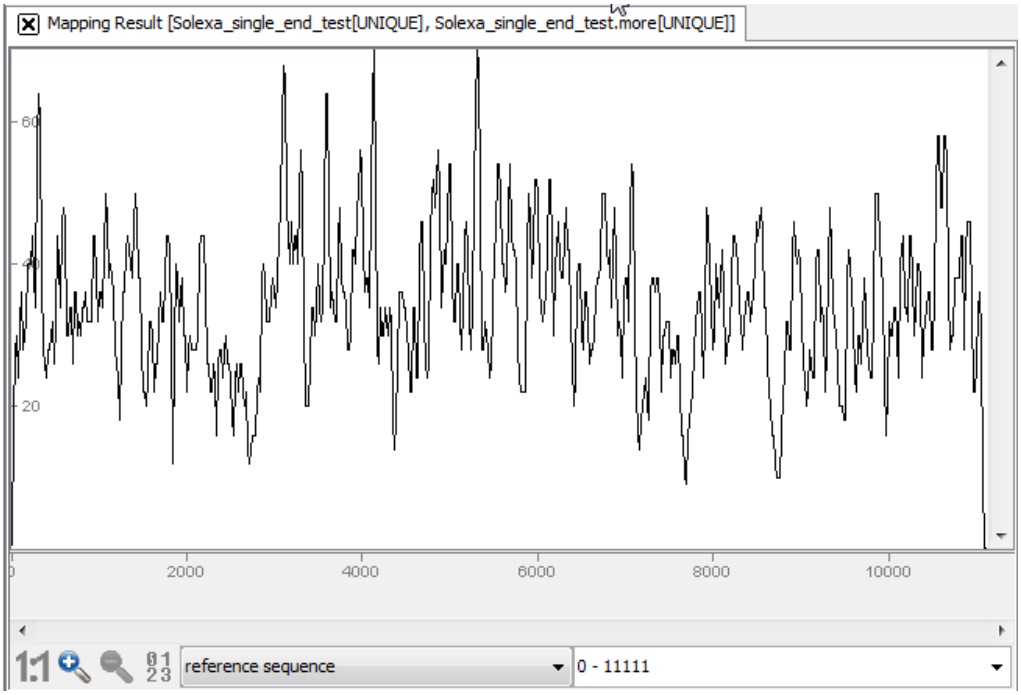
3.10 Show Mapping Results of Several Jobs Together

If two or more jobs have the same reference sequence, you can choose to merge the mapping results of these jobs to show the mapping results together.

1. Press the "Ctrl" key on the keyboard, and click the "Solexa_single_end_test[UNIQUE]" Results node and the "Solexa_sinlge_end.more[UNIQUE]" Results node. Release the "Ctrl" key.



2. Click the " " toolbar icon, to display the merged mapping results in the "mapping results window".

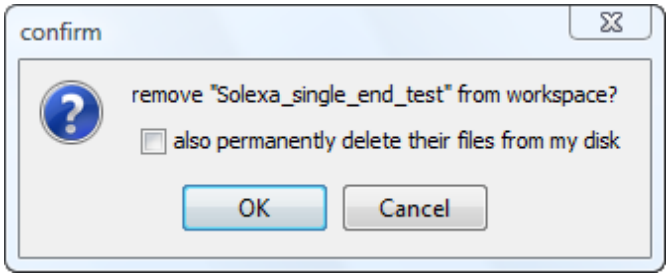


You can do any operation on it as single result node, or SNP finding on these merged mapping results.

3.11 Remove jobs

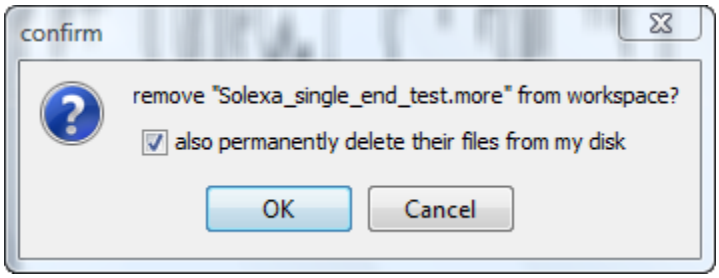
If you want to remove jobs from the workspace or disk, click the corresponding job nodes, and then click the " " tool bar icon.

1. Click on the "Solexa_single_end_test" job node and click the " " tool bar icon. A confirming dialog will pop up:



Press "OK". The "Solexa_single_end_test" job node will be removed from the "Job View" panel. This operation will only remove the job node from the "Job View" panel. You can click the " " open icon, and select the directory where "Solexa_single_end_test" is stored to load the job into your workspace again.

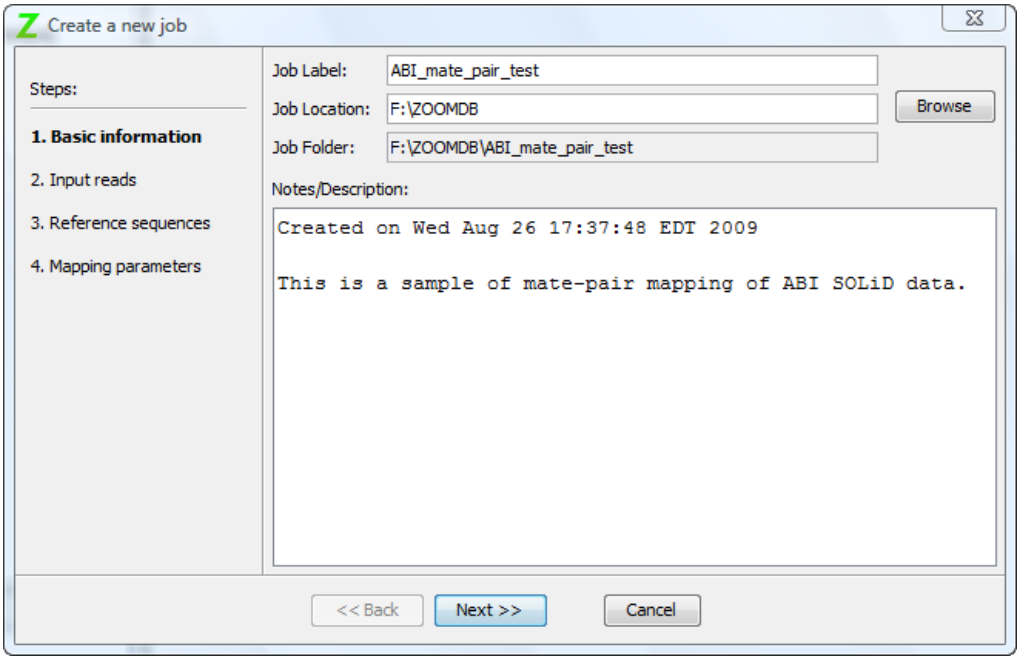
2. Click on the "Solexa_single_end_test.more" job node and click the " " tool bar icon. Click on the checkbox and press "OK". All the items related to the job including the directory on the disk will be deleted permanently.

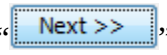


3.12 Paired-end/Mate-pair read mapping example

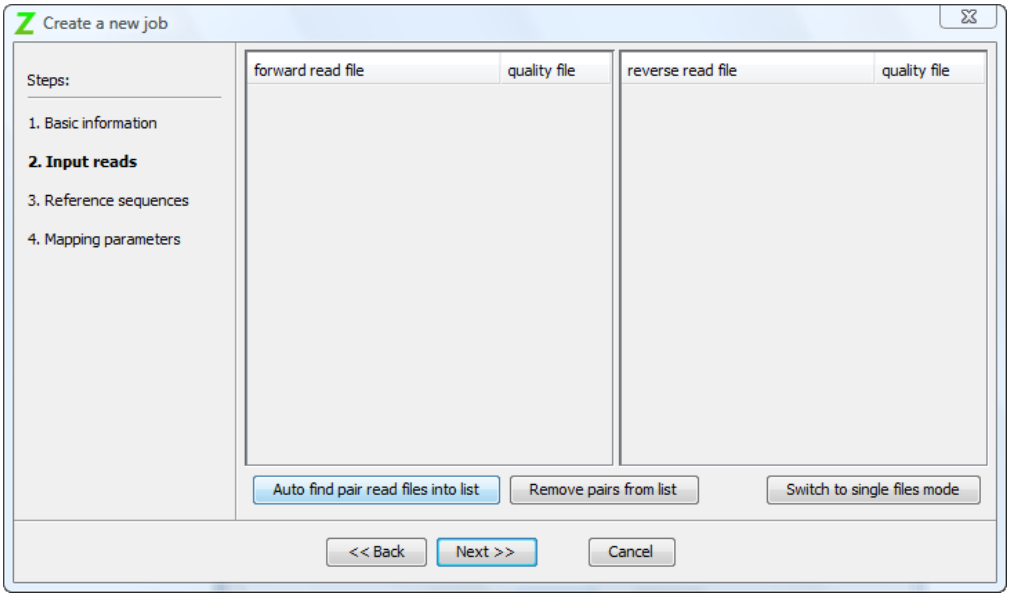
We assume that you have gone through the above single-end reads mapping process. Now we will explain how to map paired-end/mate-pair reads, focusing only on the operations that are different from mapping single-end reads.

1. Click “ ” to create a new job named “ABI_mate_pair_test” as follows:



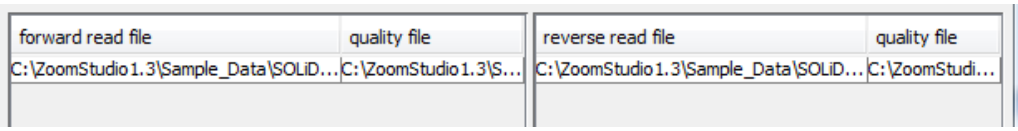
2. Click the “  ” button to move to the “Input reads” step.

Click “ ” to change to the mode of inputting mate-pair reads as follows:



The read file list window is split into two windows, each window load each end of the mate-pair reads file. Make sure every two files in the same row of the left and the right window are paired.

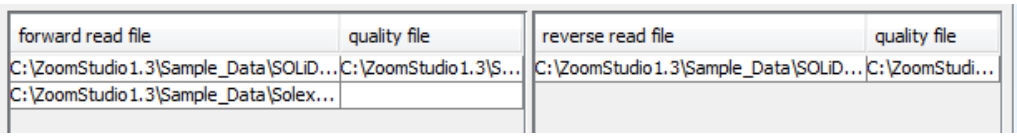
3. Click the "Auto find pair read files into list" button. Choose both "read_F3.csfasta" and "read_R3.csfasta" files in "Sample_Data\SOLiD\mate-pair\" directory. ZOOM will automatically recognize the possible paired files and put them in one row together with their quality file if any.



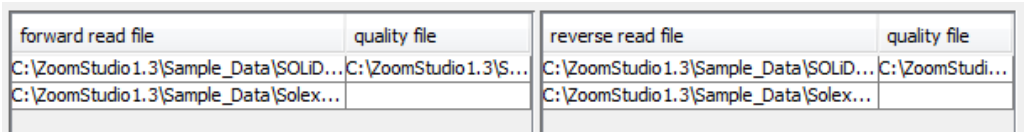
ZOOM automatically finds paired read files according to the suffix of the files: *<filename>_F3.csfasta* will be paired with *<filename>_R3.csfasta*; and *<filename>_1.fastq* will be paired with *<filename>_2.fastq*.

If you choose a directory, ZOOM will automatically pair the files satisfying the naming rule. Thus if you want ZOOM to pair the read files for you, please make sure the file suffixes are correct. You can choose to add some patterns of recognizing paired-end files as described in Section 4.10. Otherwise, you will need to feed in the reads files one pair by one pair by yourself as follows:

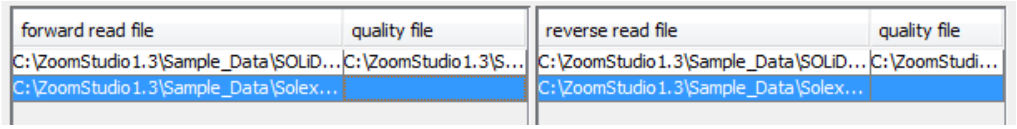
- Double click the left "forward read file" window, and select the "Sample_Data\Solexa\pair-end\read_1.fastq".



- Double click the right "reverse read file" window, and select the "Sample_Data\Solexa\pair-end\read_2.fastq".

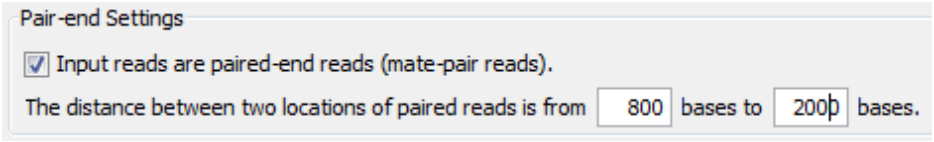


PLEASE KEEP IN MIND that two reads files in one row are paired. When you select one file, the two files in the row are both selected as follows:

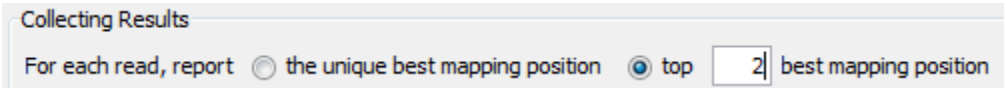


- Select the Solexa data and click the "Remove pairs from list" button to delete the file. We will not be using this set of data in the following tour.

4. Click "Next >>" to move on to the "Reference sequences". Choose the "reference.fa" file in the ""Sample_Data\SOLiD\mate-pair\" directory. Click "Next >>" to move on to the "Mapping Parameters".
5. The estimated range of the distance between two reads of one mate-pair is [800, 2000]. Set the paired-end parameters as follows:



Keep the top two mapping results for each read:



Click "Finish" to create the "ABI_mate_pair_test" job.

6. After the job is finished, click the "ABI_mate_pair_test[UNIQUE]" result node and click the "" toolbar icon to show the mapping results.
7. Click any place in the "Mapping Results Illustrating window", select a read, and press the "Find its mate-pair" button. ZOOM will then jump to the pair of the selected read.

Chapter

4

Load Data

4. Data Format

Before loading any data files into ZOOM, please make sure that the data is in an acceptable format.

ZOOM accepts reads from both Illumina/Solexa data and ABI SOLiD color space data. ZOOM can handle data files in the following formats:

4.1 Illumina data

ZOOM accepts five types of Illumina/Solexa read files as input. These file formats are automatically recognized. The letters of the read sequences are case insensitive. The length of the reads can be different.

FASTA format

Example of FASTA format:

```
>read1A_1

AGGACTATATTGCTCTAATAAATTTGCCGGTTCTTA

>read1A_2

TCTAATAAATTTGCCGGTTCTTAAAAACTCAAT

>read1A_3
```

In ZOOM, FASTA format files have no sequencing quality scores, thus all the read bases including N are considered equally relevant.

****_seq.txt and *_prb.txt Files***

Please put the *_seq.txt and *_prb.txt in the same directory. ZOOM will pair the <filename>_seq.txt and <filename>_prb.txt automatically.

Example of *_seq.txt file format:

```
1      1      125    701 GCTACCCTTTAGGTTTAA
```

Each line of the sequence file records the channel number, tile number, x position, y position of each sequence read, and the sequence of the read. The labels of each read sequence are in the format of *<channel number>_<tile number>_<x position>_<y position>*.

Example of *_prb.txt file format:

```
40 -40 -40 -40 40 -40 -40 -40 40 -40 -40 -40 40 -40 -40 -40...
```

The *_prb.txt file contains the quality score of each possible nucleotide base for the given cycle number. Four numbers, such as *-40 40 -40 -40*, each separated by a space, are the sequencing quality scores associated for each possible nucleotide, *ACGT*, respectively. The tab character is used to separate the bases of each cycle. Each line of *.prb is associated with the corresponding line of *_seq.txt.

****_prb.txt Files***

Example file:

```
40 -40 -40 -40 40 -40 -40 -40 40 -40 -40 -40 40 -40 -40 -40...
```

*_prb.txt file is the same as the description in part 2 above. ZOOM can process this file without corresponding *_seq.txt file as input. The difference is that the labels of each read sequence are automatically assigned.

FASTQ Format

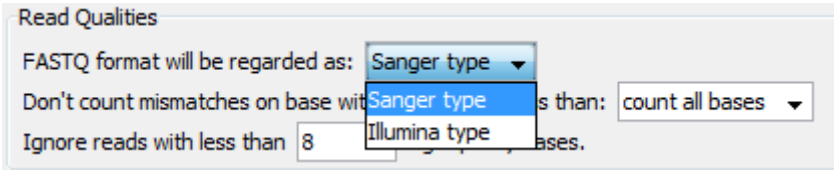
ZOOM accepts FASTQ format, where four lines represent one read in the following format:

```
@read_name

read sequence
```

Example of FASTQ format:

```
@071113_EAS56_0053:1:1:756:463

GTGATTAGTGAAACATAAAATAGTTTCATGTTGAAA

+

IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIGIAI

@071113_EAS56_0053:1:1:813:752
```

The FASTQ format includes Sanger FASTQ format and the Illumina/Solexa FASTQ format, which scale differently. For Sanger FASTQ format, the quality score of each position equals ord($q)-33, while for Illumina/Solexa FASTQ format, the quality score of each position equals ord($q)-64. When creating a job, ZOOM has a combo box in the parameter selection part to assign whether the FASTQ format is Sanger FASTQ format or the Illumina/Solexa FASTQ format.



One read per line with quality scores

Example format:

```
4_87_872_656 ACGTACNT 40 40 40 40 35 60 0 70
```

The first column contains the read sequence label or description, the second column contains the sequence data of the read, and the third column contains the quality scores for each base. Note that the read sequence label or description should not have a space inside.

4.2 ABI SOLiD color space data

Applied Biosystems SOLiD *.csfasta File

ABI SOLiD represents their reads in color spaces. Any two adjacent nucleotide bases from the read are represented by one of four colors. The mapping relationship between base space (nucleotide) and color space is denoted in the definitions section under "color space".

In this release, ZOOM accepts the color space data (*.csfasta) from SOLiD, in which each read is a numeric string prefixed by a single base. The base that precedes the numeric (color code) data is the final base of the sequencing adapter.

Example of *.csfasta file format:

```
>1_6_678_F3

T00300110000021203222220223

>1_6_1142_F3

T10110103213131233210222222

>1_6_1616_F3

T22200122131213222231133320
```

Applied Biosystems SOLiD *.csfasta and * _QV.qual File

ABI SOLiD data stores color space sequence of reads in *.csfasta file and corresponding quality score of each read in *_QV.qual file. Note that ZOOM load the quality score file along with the *.csfasta file automatically. So please put the *.csfasta file and the *_QV.qual file together in one directory and set the prefix file name the same.

Example of *_QV.qual file of the above *.csfasta:

```
>1_6_678_F3

10 8 24 10 14 8 11 10 5 8 5 10 5 7 2 3 9 2 3 5 2 7 4 4 5

>1_6_1142_F3

8 11 8 17 14 8 25 20 15 14 16 17 11 10 19 16 25 15 5 16 13 19 10 6
12

>1_6_1616_F3

2 11 8 8 6 7 3 4 11 8 5 16 8 10 11 6 3 14 16 9 5 19 7 10 8

>1_6_1634_F3
```

Applied Biosystems SOLiD *.fastaq File

The contents in *.csfasta and *_QV.qual can be integrated as a CSFASTQ file. It is similar to the FASTQ format, where four lines represent one read. The only difference is that the read sequence is in color space format.

```
@read_name

read sequence
```

The quality score of each position is denoted by a nucleotide. The numerical score is ord($q)-33.

Example of CSFASTQ format:

```
@SRR015241.1 CLARA_20071207_2_CelmonAmp7797_16bit_26_88_34_F3 length=50

T32322133300002330031001022230020232002203222030231

+SRR015241.1 CLARA_20071207_2_CelmonAmp7797_16bit_26_88_34_F3 length=50

!21(()+%'+%40*.%%**)&%&*&%%%&%%%%%%%%%%%%%%%(+%%%%'
```

There are two sequences in the above two example. Note that the first character of the quality score string will be viewed as the quality of the adapter nucleotide.

4.3 Reference sequence file format

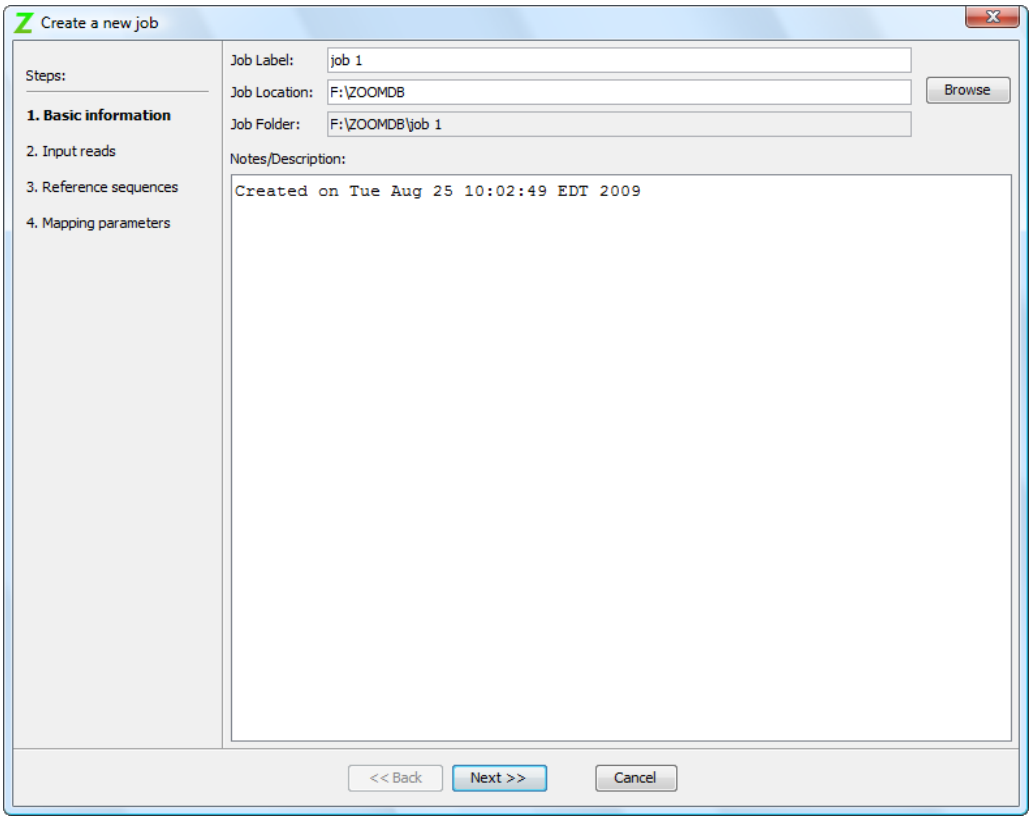
ZOOM accepts reference sequence files containing reference sequences in FASTA format.

Example format of a reference sequence file:

```
>Reference_sequence_1_name

AGGACTATATTGCTCTAATAAATTTGCGGTTCTTAAAAACTCAATGT

TGTAAAAATGTCACTTCTTCCCAAA…
```

4.4 Create a new Job

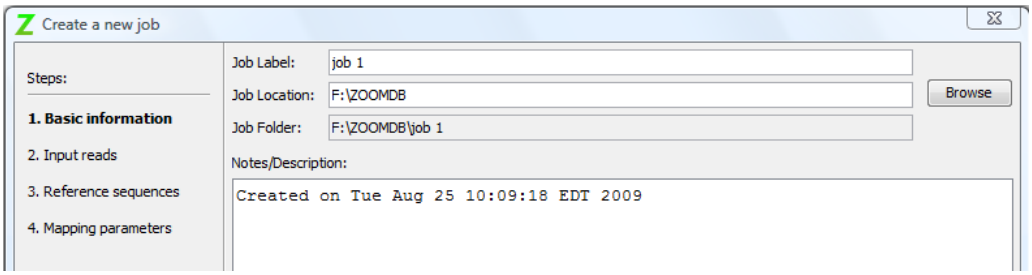
To create a new project, click on the “Create a new job” toolbar icon “ ” or select “New Job” from the “File” menu. The “Create a new job” window will open as follows:



There are four steps to create a job.

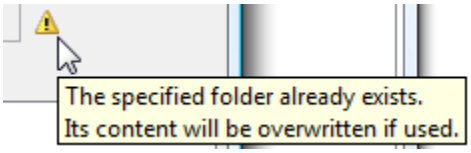
Basic information

This section is used to assign a name for your job and a directory to store the data related to your job. After you have finished the job, you can load the job to show the mapping results or perform post-analysis by selecting the directory.



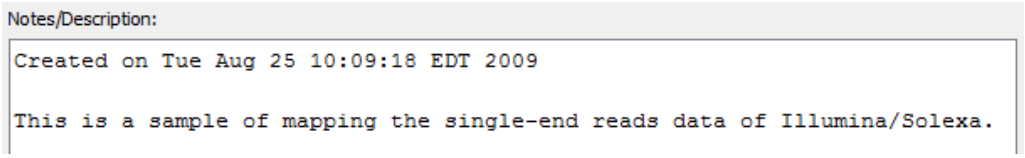
1. Enter a name for your job in the blank field beside the "Job Label":

If the job name you input already exists in the directory, there will be a tool tip icon below the Browse button as follows:



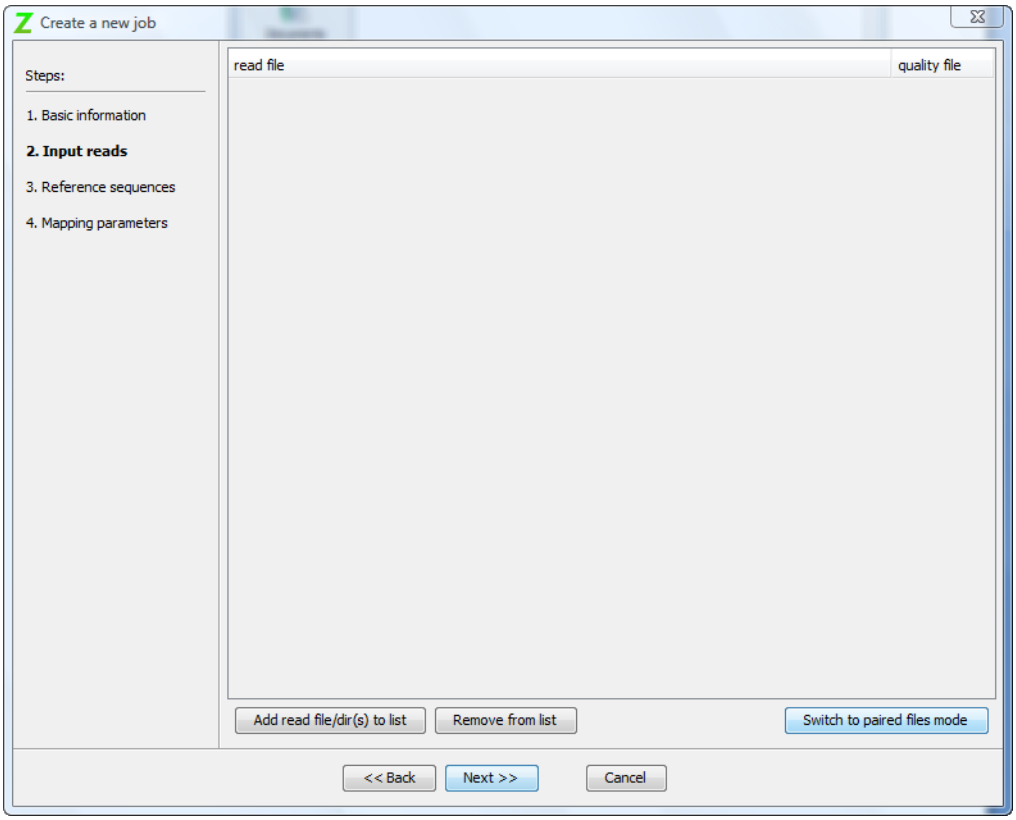
You can choose to change the job name or to overwrite the existing directory.

2. Press the "Browse" button. Select a directory to save your job.
3. You can enter any descriptions about your job for future reference.


Notes/Description:
Created on Tue Aug 25 10:09:18 EDT 2009
This is a sample of mapping the single-end reads data of Illumina/Solexa.

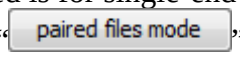
4. Click the "Next >>" button at the bottom of the window.

Input reads



All reads data are input here.

There are two ways to input reads files: by selecting read files or directories. ZOOM will automatically search for all the reads files inside. Please note that the read file should be in a standard format of next generation sequencing technologies. For example, "*_seq.txt", "*_qual.txt", "*.fastq", "*.fasta" files for the Illumina data, "*.csfasta" "*_QV.qual" "*.fastq" files for ABI SOLiD data, as described in Section **Error! Reference source not found.**.

The default "Input reads" window opened is for single-end reads. To change to the "paired-end/mate-pair reads" window, click the " 

" button. Click it again to switch back to the single-end reads input mode.

- Input single-end reads

1. Click the "Add read file/dir(s) to list" button. Choose the read file(s) or directories which contain the read files. By default, ZOOM will find all the files suffixed by "*_seq.txt", "*_qual.txt", "*.fastq", "*.fasta", " *.csfasta " " *_QV.qual" files to load in as the reads file.

2. To remove the files you don't want, select the files and click the "Remove from list" button. The files will be removed.

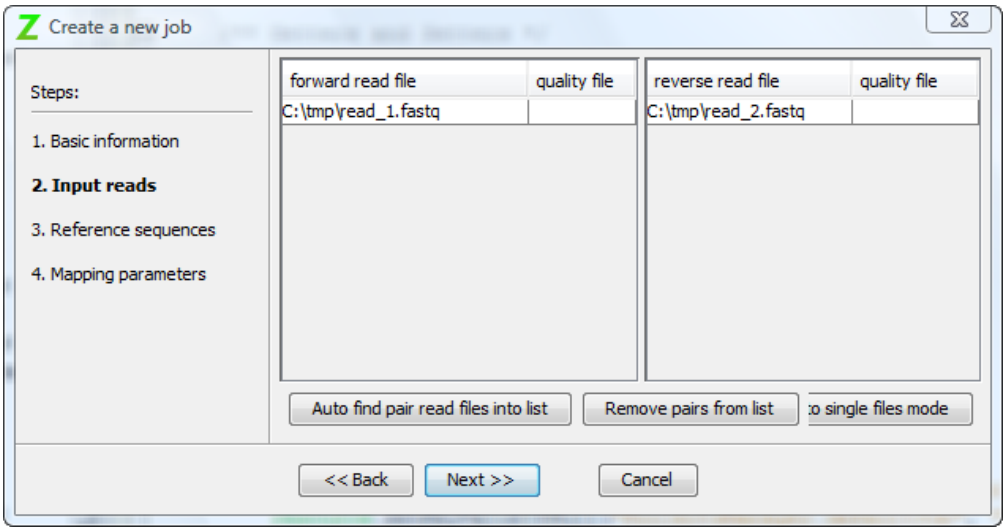
3. To add more files, click "Add read file/dir(s) to list" again as step 1).

4. Click the "Next >>" button to move on to the reference sequence selection window.

- Input paired-end reads or mate-pair reads

By default, the two mates of one pair are stored in two files separately, but you are allowed to store the two mates of one pair next to each other in one file. However, in that case, please use single-end reads mode to load reads and assign mapping in paired-end mode in the "Mapping Parameters" section.

1. Click the "paired files mode" button to change to paired-end reads input mode. The read list window is split into two windows, each of which is for loading the file containing one mate of a pair.



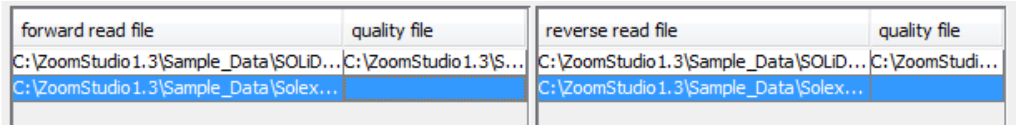
2. Click the "Auto find pair read files into list" button and select the read files or directories. ZOOM will automatically pair these read files according to the file names. The paired file will be on the same row of the two windows. ZOOM recognizes the read file pairs according to the following file name patterns:

- <filename>_1.fastq and <filename>_2.fastq
- <filename>_F3.fastq and <filename>_R3.fastq

If you choose a directory, ZOOM will automatically pair the files satisfying the naming rule. If you want ZOOM to pair the reads for you, please make sure the suffixes are correct. Otherwise, you will need to upload the reads file pair by pair by yourself as follows:

- Double click the left "forward read file" window, and select one file <file1>.
- Double click the right "reverse read file" window, and select the other file <file2>. **MAKE SURE** that <file2> contains the mates of the reads in <file1>, since the two files will be in the same row in the two windows and be treated as read pairs.

KEEP IN MIND that reads file in one row are paired. When you select one file, the two files in the row are selected as follows:



3. To remove read pair files, select the files and click the "Remove pairs from list" button to delete the files.

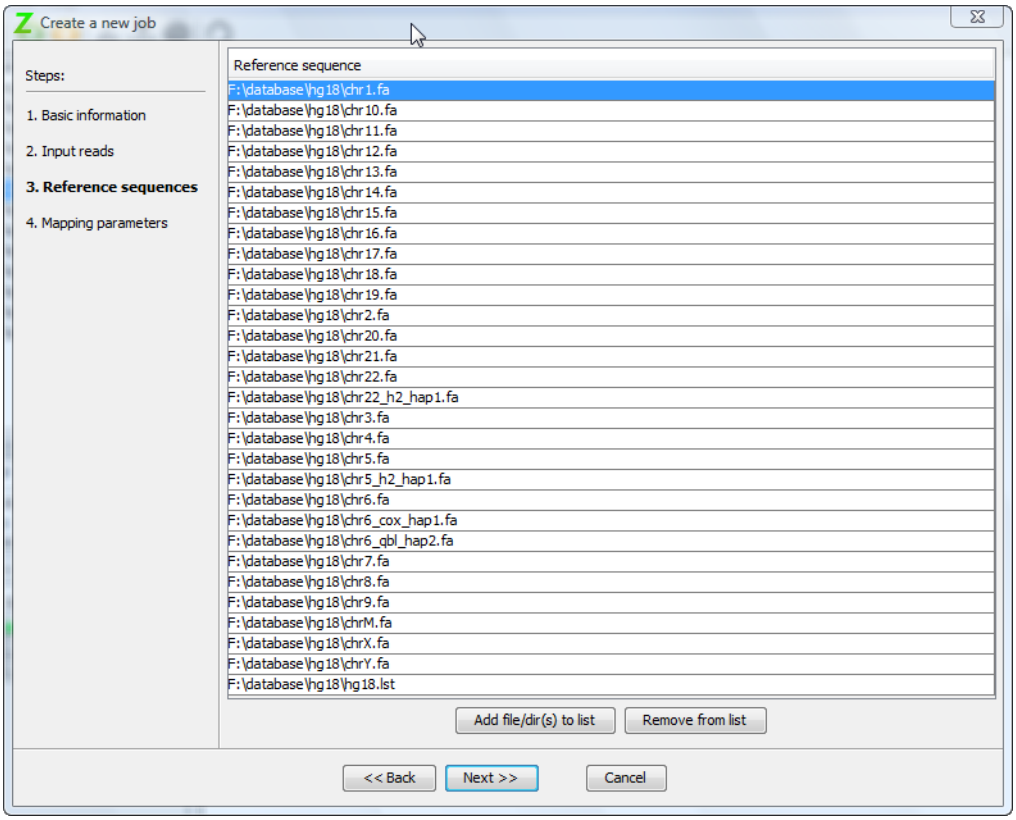
4. To add more read pair files, click "Auto find pair read files into list" or double click the two windows as above.

5. Click the "Next >>" button to the reference sequence selection.

Reference sequences

Assign the reference sequences where the reads data are to be mapped. The sequences in the reference file should be in FASTA format. Multiple reference files or a directory can be loaded into ZOOM.

1. Press the "Add file/dir(s) to list" button, and choose multiple files or directories. If directories are chosen, all FASTA files in this directory will be loaded there.

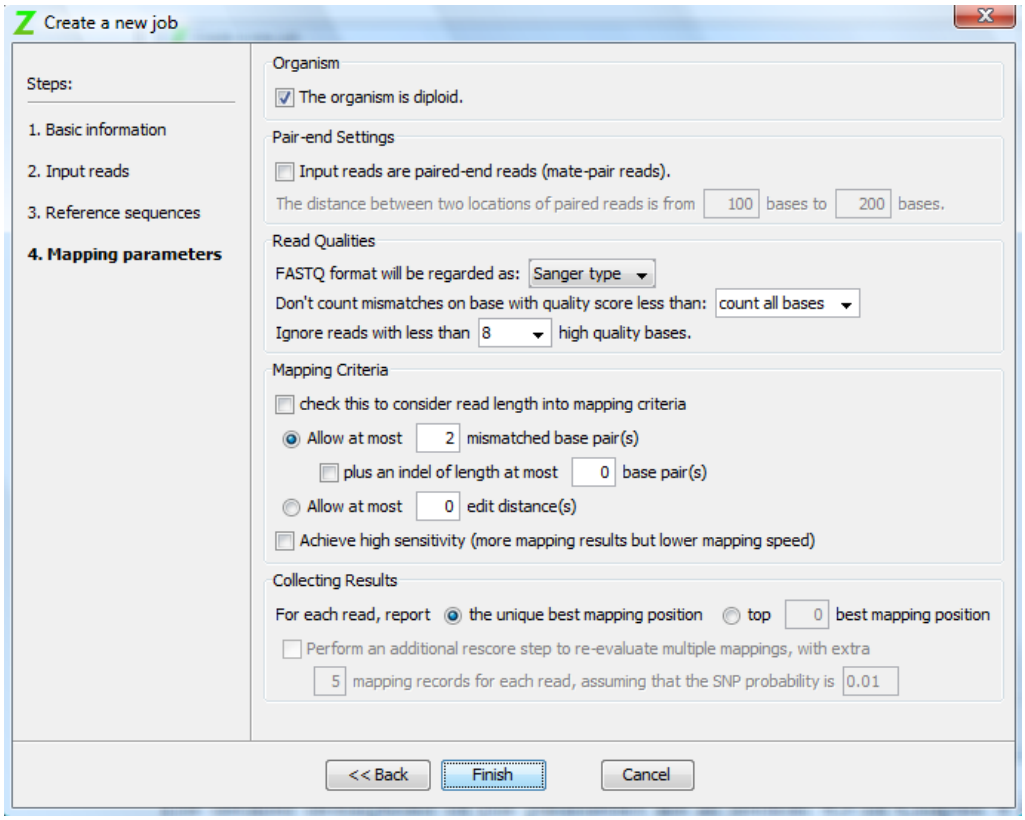


2. To remove some reference files, click these reference sequences and click the "Remove from list" button to remove these files.

3. Click the "Next >>" button at the bottom of the window to proceed to the "Mapping parameters" window.

Mapping parameters

There are five groups of parameters in the following "Mapping parameters" window. The five groups of parameters will be explained in next section. After choosing the proper parameters, please press the "Finish" button to create the job.

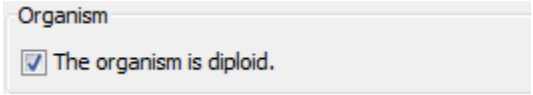

Create a new job
Steps:
1. Basic information
2. Input reads
3. Reference sequences
4. Mapping parameters
Organism
The organism is diploid.
Pair-end Settings
Input reads are paired-end reads (mate-pair reads).
The distance between two locations of paired reads is from 100 bases to 200 bases.
Read Qualities
FASTQ format will be regarded as: Sanger type
Don't count mismatches on base with quality score less than: count all bases
Ignore reads with less than 8 high quality bases.
Mapping Criteria
check this to consider read length into mapping criteria
Allow at most 2 mismatched base pair(s)
plus an indel of length at most 0 base pair(s)
Allow at most 0 edit distance(s)
Achieve high sensitivity (more mapping results but lower mapping speed)
Collecting Results
For each read, report the unique best mapping position top 0 best mapping position
Perform an additional rescore step to re-evaluate multiple mappings, with extra
5 mapping records for each read, assuming that the SNP probability is 0.01
<< Back
Finish
Cancel

A “Processing” window will pop up. The reads in the read files will be loaded. After this process is finished, a job is created inside the directory you assigned. All the information about this job is stored in this directory. You can copy this directory anywhere. If you use ZOOM to load this directory, the job can be shown and post-analysis can be carried out on it. You can inspect the status of this job in the “Job view window”.

4.5 Parameters

There are five groups of parameters that you can select from as prefered.

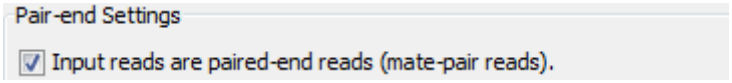
Organism



This is a checkbox to decide whether the organism is diploid. When the option is selected, ZOOM will assemble the consensus sequence as a diploid genome. Bases in the consensus sequence will be presented using the IUPAC code.

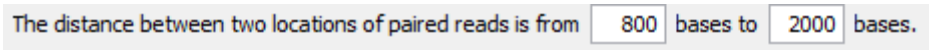
Pair-end Settings

Check the box when you want to align reads in paired-end mode.



If you check the box and there are no read files added in "paired file mode", ZOOM will treat every two sequences in the file added in "single file mode" as the two mates of a pair.

When selecting this box, please remember to assign the distance range between the two reads that make up the read pairs as follows:

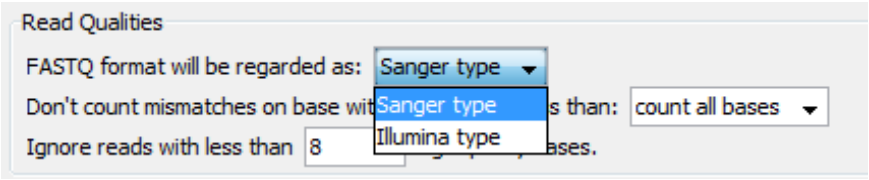


Read Qualities

Quality score of read reflects the sequencing quality of each base. The quality score of each base denotes the probability that this base is correctly sequenced. By default, ZOOM displays the quality scores along with the alignment between the read sequence and its target region on the reference sequence, to give the users an intuitive impression about which bases have low quality score., as follows:

Quality score can be utilized to enhance the mapping results as well.

If the reads files are in FASTQ format, according to the different methods of coding numerical quality score into alphabet, there are two types of FASTQ format ------ "Sanger type" and "Illumina type". For Sanger FASTQ format, the quality score of each position equals ord($q)-33, while for Illumina/Solexa FASTQ format, the quality score of each position equals ord($q)-64.

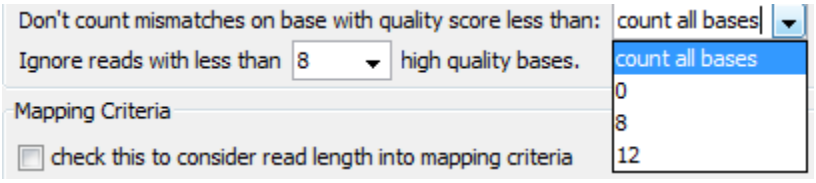


Choose the correct type by clicking the combo box.

ZOOM adopts two ways to utilize quality scores to enhance mapping results.

The first way is to ignore mismatches occurring on read positions with low quality scores during the mapping process.

A low confidence score denotes low sequencing quality at that position in the read. Therefore, mismatches occurring at positions with low quality scores are more likely due to sequencing error. Thus mismatches on positions with high quality scores are more meaningful than those at low quality score positions. Use the following combo box or enter some value to assign the threshold of high quality score.



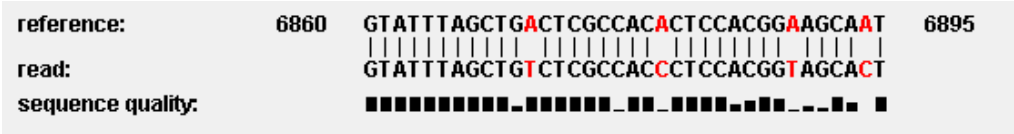
The second way is to utilize quality scores to rank several possible mapping results of each read and choose the best position as the mapping results. Since the process happens after all mapping positions have been found, this way will be described in part "5. Collecting Results".

In our experiments, we observed that more reads were uniquely mapped when quality scores were included to help mapping.

Mapping Criteria

Mismatches and insertion/deletion

The following is an example that a read is mapped to the reference sequence with only mismatches. The four red characters in the alignment are the mismatches between the read and its target region on the reference sequence.

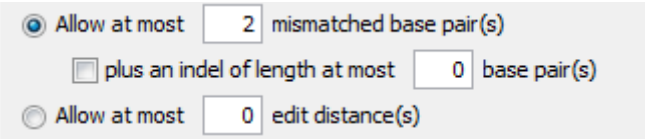


The following is an example that a read is mapped to the reference sequence with a deletion on the read. The read deletes an "A" nucleotide. There is one deletion of length one.

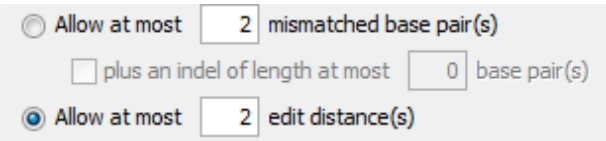
The following is an example that a read is mapped to the reference sequence with an insertion of length one. The red "A" base is the insertion. There are two mismatches and one insertion of length one.

ZOOM will map reads to the target regions on the reference sequence within a given mismatch number or insertion /deletion length.

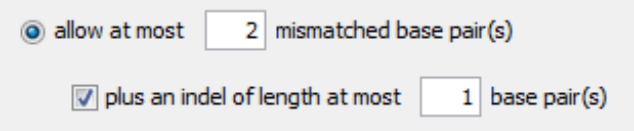
- If only mismatches are allowed between reads and reference sequences, use



- If you want to allow insertion and deletions, you can use edit distance too. The edit distance is the addition of the number of mismatches and the length of the insertion / deletion.



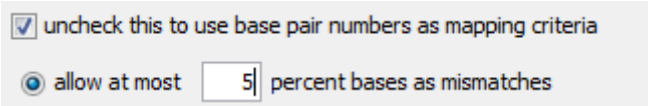
- If you want to allow indels AND you want to assign the length of the indel, check the radio box and the check box and assign the number you want as follows:



This will allow up to two mismatches and one insertion/deletion of length one between the reads and the reference sequence.

You can choose to assign a ratio of mismatches to read length instead of the mismatch number by checking the following checking box:

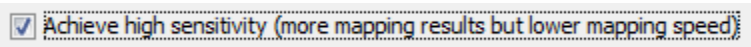
Assign the ratio of mismatches of the read length.



Commonly, the ratio criterion is useful for those read files containing reads of various lengths.

Getting High Sensitivity Results

ZOOM adopts the optimal multiple seeds strategy to guarantee 100% sensitivity for a wide range of read lengths and mismatch numbers. However, using these seeds might be time-consuming especially for very long reference sequences. By default, ZOOM adopts the seeds guaranteeing 100% sensitivity to find all mapping positions having up to 2 mismatches with the reads. To get high sensitivity, please click the following check box.



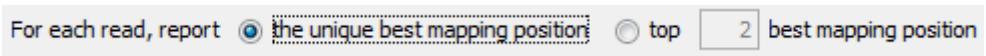
Then ZOOM will select the seeds with high sensitivity according to the mismatch number you assigned. For cases where ZOOM can achieve 100% sensitivity, please refer to Chapter 8.

Note that this option might be time-consuming when your reference sequences are long. One way to enhance the speed is to map reads without selecting this option first, then extract those unmapped reads to utilize this option. Please refer to Section 4.7 for more information.

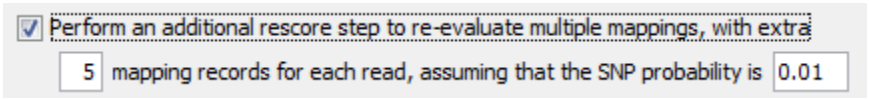
Collecting Results

Each read may be mapped to multiple target regions in the reference sequence. The best mapping results of one read are the ones with the smallest edit distance, or in case of equal edit distance, the shortest insertion/deletion length (under the consideration that insertion/deletions are less probable than mutations). If there is only one such best mapping result for the read, this is a uniquely mapped read. Otherwise, if there are multiple such mappings, the read will be considered ambiguously mapped.

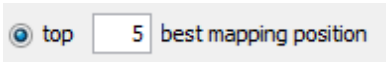
ZOOM finds all possible mapping positions satisfying the mapping criteria for each read. However, you can choose to reserve the uniquely mapped reads or the top N mapping results for each read. Choose the following radio box to switch between the two modes.

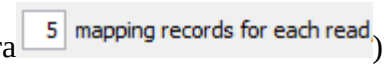


You can utilize quality scores to assess the mapping probability of possible mapping results of each read and rank the mapping positions for each read according to the mapping probability scores by clicking the following checkbox:


Perform an additional rescore step to re-evaluate multiple mappings, with extra
5
mapping records for each read, assuming that the SNP probability is
0.01

Note that only if top N mapping results are chosen, this option is available.

ZOOM adopts a re-score scheme. First, it finds all possible mapping positions of each read within the mismatch threshold or edit distance threshold. Then it picks the multiple positions (the addition of 

and extra 

) to compute the probability of the alignments between the reads and these target regions. The mapping scores (log10 (probability of the alignment)) are sorted to get the top N results as mapping results. In our experiments, more reads can be uniquely mapped after re-scoring using quality scores.

The rescore function for the ABI SOLiD data has used the priori SNP probability of the organism, therefore if the data set is ABI SOLiD data, please assign a priori SNP probability of the organism. For example, for Human, this value can be set as 0.001.

4.6 Open a Job

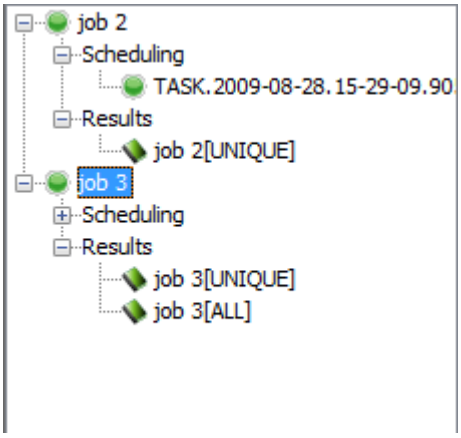
1. To open an existing job, select “Load Job” from the file menu or using the “Load job” icon “ ” on the toolbar.
2. Select the location of the data for that job.
3. The job will be loaded in the Job View Panel. You can continue to rerun the job or analyze mapping results for those finished jobs.

This option is useful when you want to close the client windows. After jobs are created, you can close the ZOOM main windows. The jobs are running on the ZOOM server. You can open the ZOOM main windows and load the job to inspect the running status or analyze the results at any time.

4.7 Orienting Yourself

Job View Panel

This frame appears in the upper left hand corner of the ZOOM main window, displaying the organization of particular jobs (if applicable). You can control these jobs and inspect the running status of the jobs in this panel.

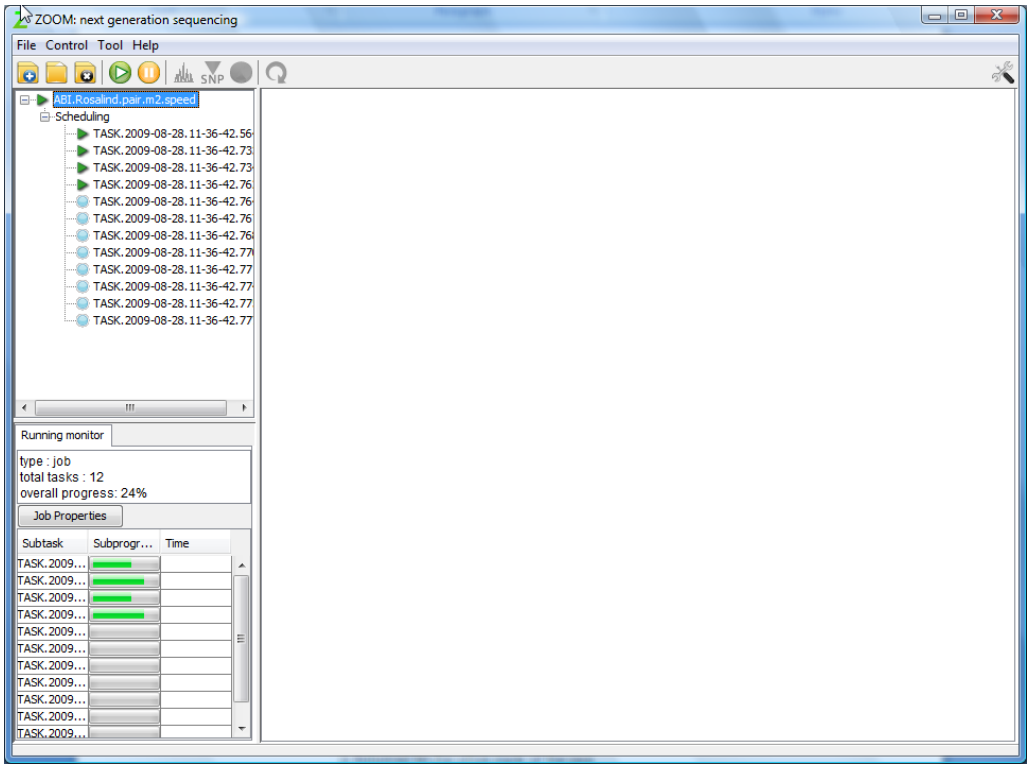


The jobs are organized as a tree. Each job is a job node having two nodes. One is a "Scheduling" node, and the other is a "Results" node. Use the '+' and '-' boxes to expand and collapse each job.

After a job is created, ZOOM will automatically create a "TASK" node under Scheduling to map these reads.

After the job is finished, the "Results" node "" will appear containing one or two results nodes to show the mapping results and carry out the post-analysis.

If the amount of reads is very large, ZOOM will automatically partition the reads into several parts and launch several tasks for each part of the reads. ZOOM will schedule these tasks automatically until all reads are handled.



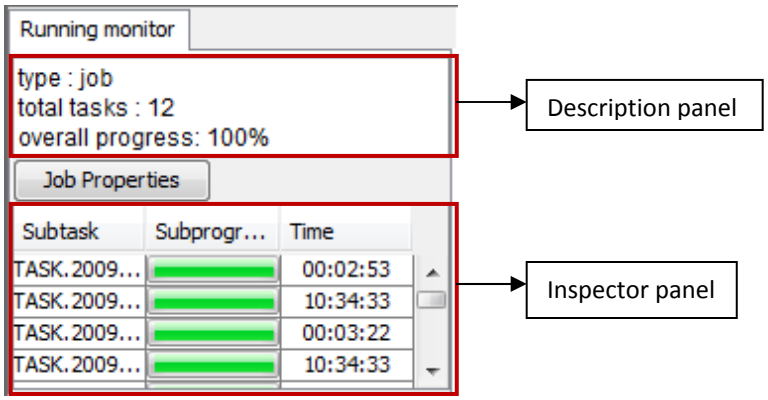
In this image, four tasks are currently running, while other tasks are waiting on the list to be scheduled.

For all the jobs created or loaded, you can view the status of the jobs and tasks using the following icons:

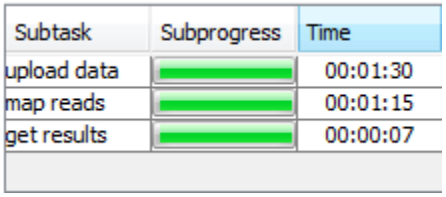
- Running icon "▶": The job or task is running.
- Canceled icon "●": The job or task is canceled.
- Error icon "●": An error occurred when the job or task was running.
- Waiting icon "●": The task is waiting to be scheduled.
- Finished icon "●": The job or the task is finished. When a job is finished, the "Results" node will appear. You can choose the [UNIQUE] or [ALL] "Results" node to show the mapping results or carry out post-analysis.

Job Running Monitor Panel

When clicking a job or a task in the "Job View Panel", the running information of this job or task will be shown in the "Job Running Monitor" below the window of "Job View Panel" as follows:



When a job is selected, the number of tasks in this job and the overall progress of this job will be shown in the "Description panel". The total number of tasks of this job and the progress of these tasks will be shown in the "Inspector panel". In the "Inspector panel", each row is the running status of a task. There are three columns denoting: the name of the task; the progress of this task; the total running time of this task (appear until the task is finished). The running time is in the format of "hours: minutes: seconds".



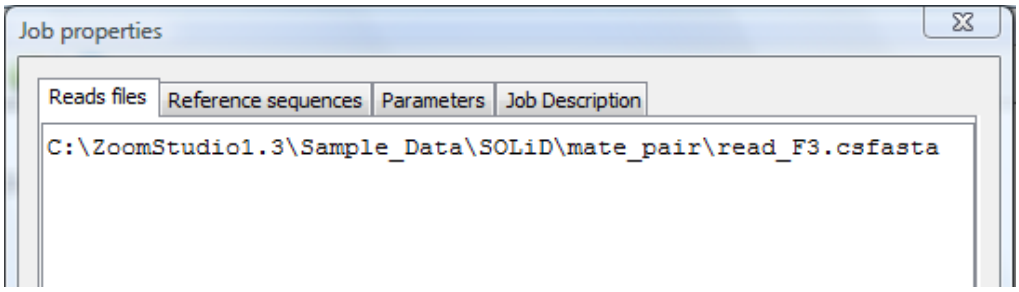
When clicking a task in the "Job View Panel", the detailed progress of each step of the task will be shown. Each row is a step of the task.

Usually, three steps are included in each task:

- Upload data- upload the reads sequences of this task to the server.
- Map reads- map the uploaded reads to the reference sequence according to the parameters assigned.
- Get results collect the mapping results from the server to the client to show the mapping results and post-analysis.

Job Properties Panel

Select a job in the "Job View Panel" and click the "Job Properties" button. The properties of this job will be shown in the popup tabbed windows.



The properties include the reads file list, reference sequence file list, the parameters used and the description note of the job.

4.8 Control jobs and tasks

There are several operations on the jobs or tasks which will change the status of the job or task:

- Rerun

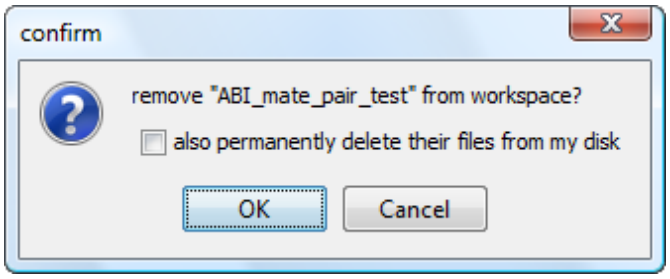
  A job canceled or having errors can be rerun by selecting the job and clicking the toolbar icon "Rerun" "". After the job is rerun, the job node icon will turn into "".

- Cancel

  If a job is still running, you can cancel it by selecting the job and clicking the toolbar icon "cancel "". The job will stop and be canceled. The icon will turn into "". Note that only running jobs can be canceled.

- Remove

  A job can be removed at any time. You can choose to remove the job from the workspace or delete all the information about the job from the computer RAM and harddrive. Select the job you want to remove and click the remove toolbar icon "". A confirming dialog will pop up as follows:



Press the “OK” button if you want to remove the job from the work space, which will result in the removal this job from the Job View Panel and the computer memory. However, you can load in the job once again at any time.

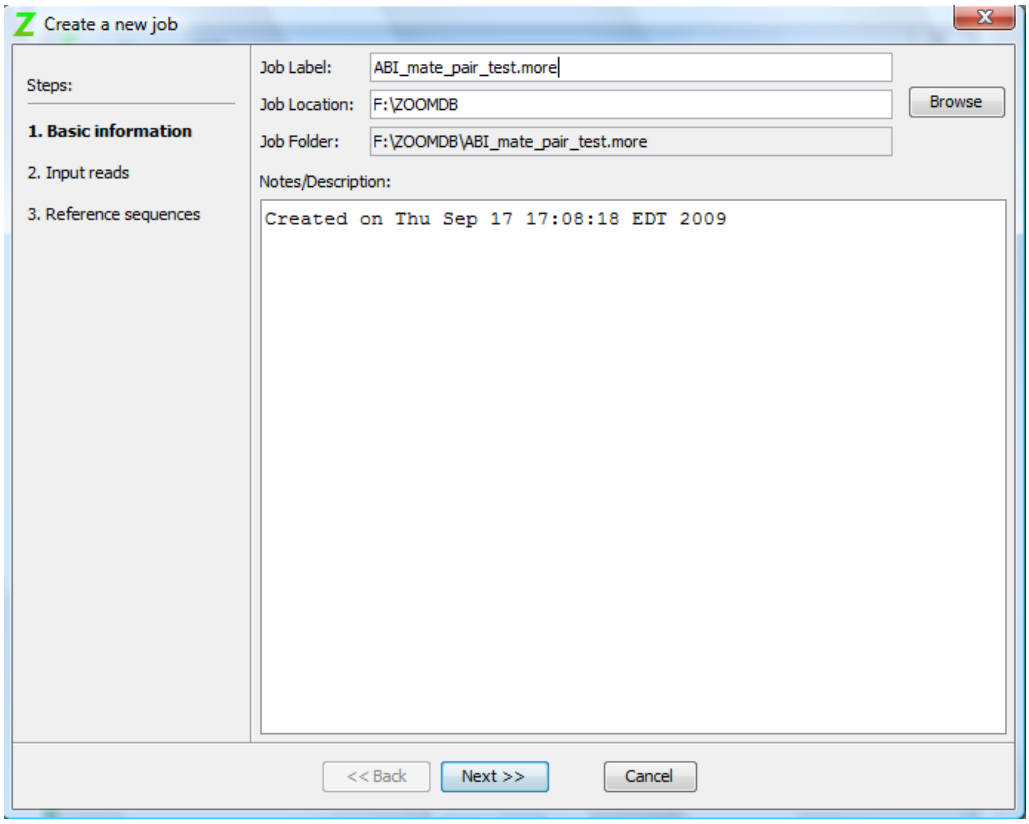
If you don’t need a particular job anymore, you can choose to delete the job from the computer. Click the check box as follows, and press “OK” button to delete all the information about the job.

4.9 Extract unmapped reads to create a new job

If you want to survey the unmapped reads of a job, you can choose to map these reads to other reference sequences, or map these reads to the same reference sequence with different parameters, for instance to allow more mismatches or edit distances.

1. Select a job
2. Press the reprocess unmapped reads toolbar icon “ ”.

The following window will pop up:



The process is similar to creating a new job whose reads data is the unmapped reads of the selected job. Assign a name to the new job for these unmapped reads. The default job name is the name of the selected job suffixed by a ".more". By pressing "Next", you can assign proper reference sequences and parameters.

There are several examples of why you might want to use this option:

- You want to find some novel transcripts from the read data you sequenced. First, you can create a job to use the known transcriptome sequence as the reference sequences and map all the reads to them. Then extract the reads unmapped to the known transcriptome, and map them to the whole human genome sequence. Those reads mapped to the whole human genome might come from novel transcripts.
- Since the mapping allowing insertion/deletion detection takes much more time than the mapping allowing only mismatches. You can map the reads allowing only zero mismatches, and then extract the unmapped reads to map with insertions/deletions. You even can choose to increase the length of insertion/deletion step by step. First, map reads with insertion/deletion length of one, then length of two as so forth. Finally show all the mapping results together. This will save a lot of running time especially when the dataset is huge and the reference sequence is long.

- ZOOM adopts the multiple spaced seeds strategy, which can guarantee to find all the alignments satisfying the mismatches threshold set by you (100% sensitivity). However, when the reference sequence is long, the process will consume more time. You can run the mapping without clicking "achieve high sensitivity" parameter first, and then extract those unmapped reads by choosing the "achieve high sensitivity" parameter later. Lastly, display the mapping results together.
- If you want to find a long insertion / deletion using mate-pair library sequencing, start by creating a job to map all the reads in paired-end mode given the range of the two mates in one pair. After the job is finished, extract the unmapped reads to align the reads in shorter or longer range of the two mates in one pair. Here you will find those candidates of insertion and deletion. You can also map these unmapped reads in single-end mode to find some translocations.

4.10 System Configuration

There are five types of configuration which will help your ZOOM run more smoothly.

Default storing directory

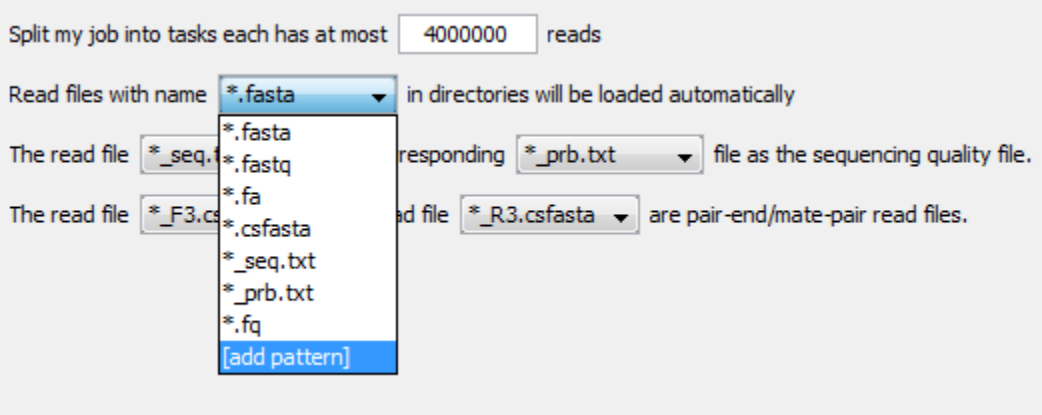
If you are used to using a directory to store your jobs, you can click "Browse" button to set the directory you preferred as the default storing directory of new created ZOOM jobs.

The size of split files

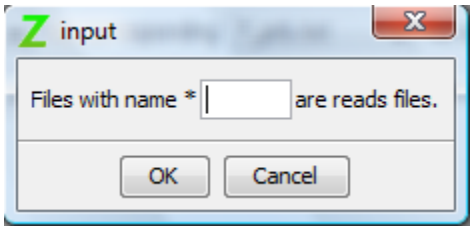
See Section 2.6.

Reads file suffix

ZOOM can automatically load read files in the directories you selected by recognizing the suffixes of the files. By default, *.fasta, *.fa, *.fastq, *.csfasta, *.fq, *_seq.txt and *_prb.txt will be loaded. If you need more patterns, click the dropdown list and choose "add pattern" as follows:



A dialog will pop up:



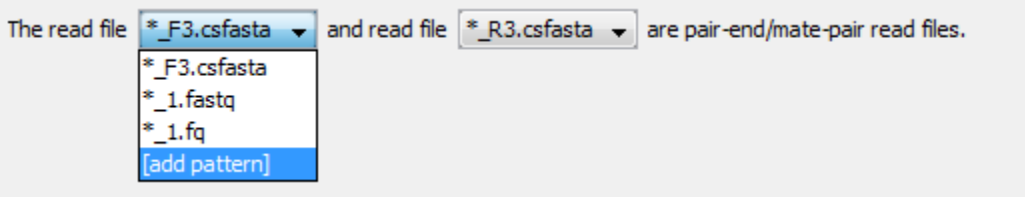
Enter the new suffix in the text field and press “OK”.

Quality score file suffix

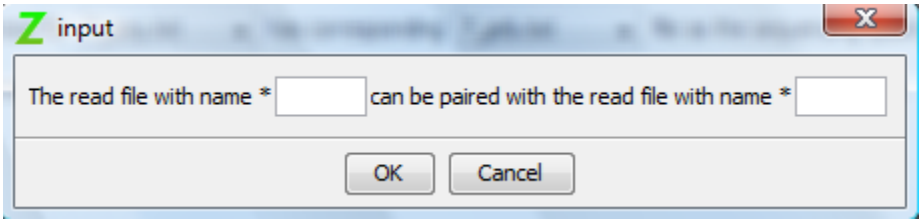
When you select reads file, its corresponding quality score file will be loaded together. By default, the quality score file of “*_seq.txt” is “*_prb.txt”; the quality score file of “*.csfasta” is “*_QV.qual” ; the quality score file of “*.csfasta” is “*.csfasta.qual”. You can add your own pattern of recognizing the quality score file.

Paired-end / Mate-pair files suffix

ZOOM can recognize the two files from the paired-end data / Mate-pair data. By default, ZOOM will mate up “*_F3.csfasta” with “*_R3.csfasta”, “*_1.fastq” with “*_2.fastq”, “*_1.fq” with “*_2.fq”. You can enter your own pairing criteria by clicking the dropdown list and choose “add pattern” as follows:



And enter the pattern you preferred in the popup windows:



Chapter

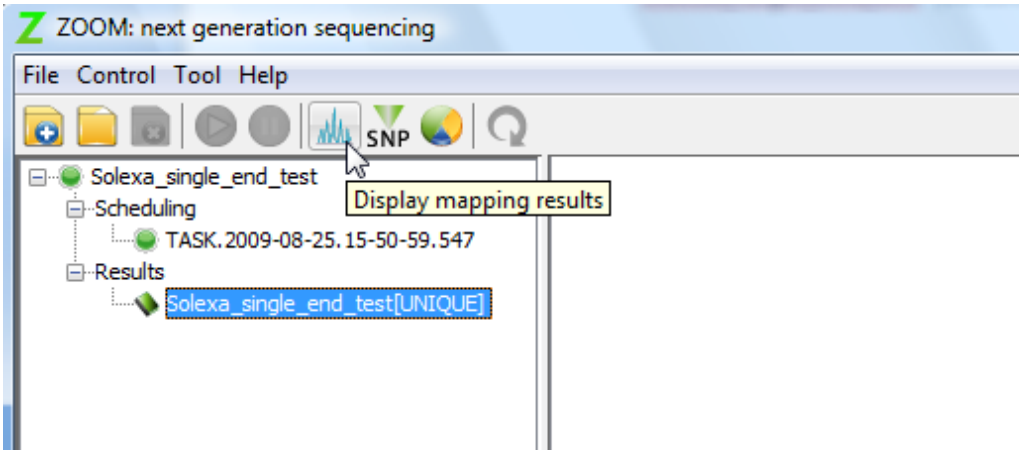
5

5. Mapping Results

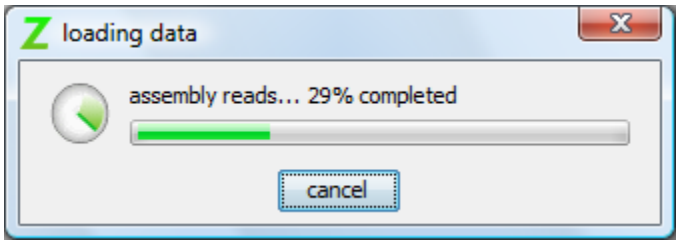
5.1 Show Mapping Results

After the job icon turns to "●", the job is finished. ZOOM will help survey the mapping results in a preferred scale. Make sure that the node under the "Results" node is selected when choosing data to be analyzed.

1. Select an "UNIQUE" or "ALL" Results node of a Job in the "Job View" panel.
2. Click the "Display mapping result" toolbar icon "".

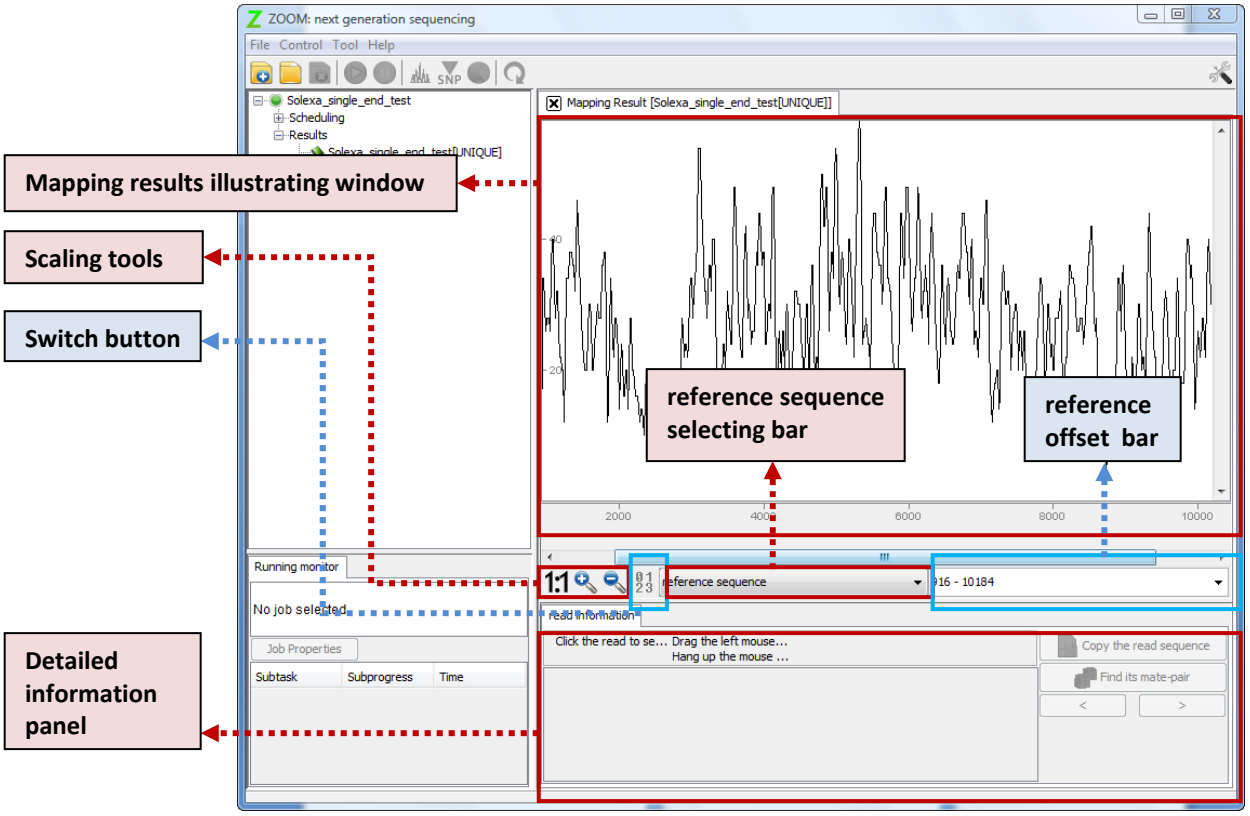


ZOOM will assemble the mapped reads into a consensus sequence and show the read depth overview along the reference sequence. This will take some time depending on the amount of mapped reads and the length of the reference sequence. The progress bar will pop up to display the progress:



By default, ZOOM will assemble the mapped reads into a consensus sequence by treating the organism as a diploid genome.

After the procedure is finished, you can see a tab window containing the mapping results on the right hand side of the main window of ZOOM as follows:

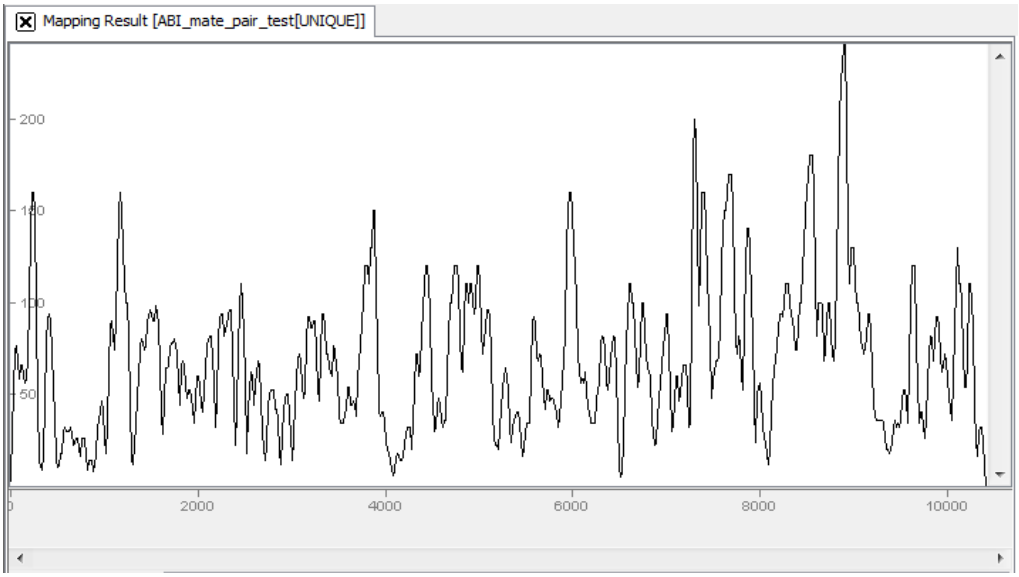


The tab window contains the six parts.

Mapping results illustrating window

This window will show the reads mapped to the whole reference sequence or specific region of the reference sequence in different scales subject to your preference. After you select a result

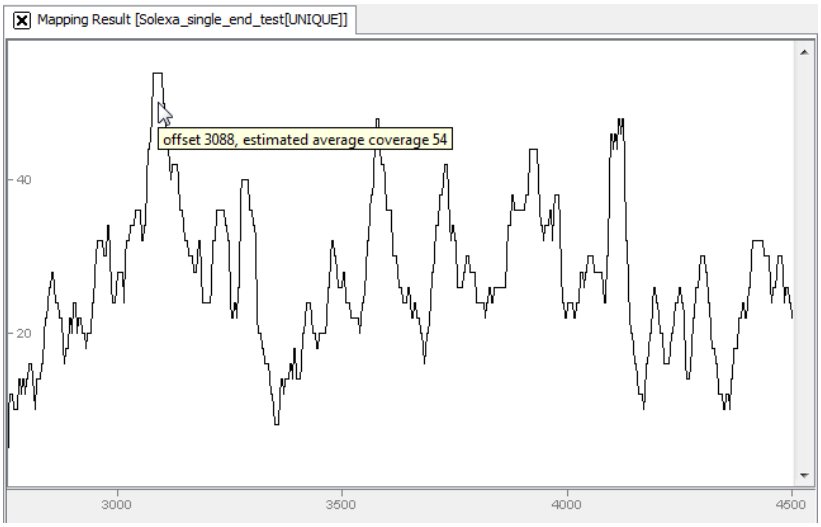
node and click the " " toolbar icon, the overview of the read depths of all reads mapped to the whole reference sequence will appear as follows:



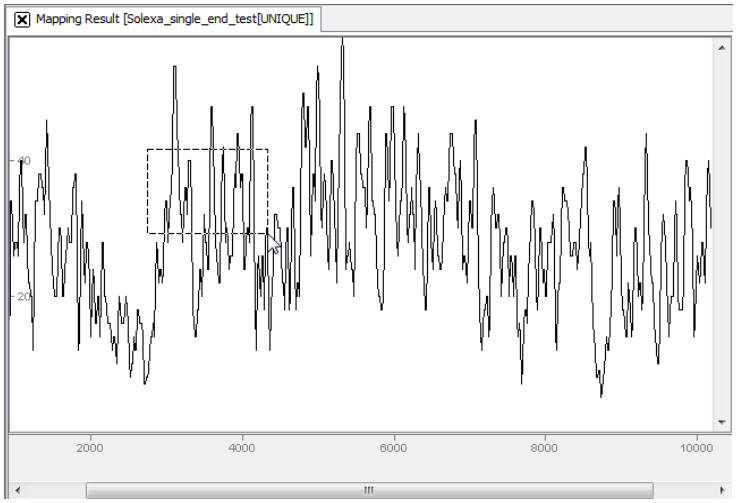
At the bottom of the window is a horizontal ruler denoting the positions on the reference sequence. The left vertical ruler denotes the read depth. You can get an idea about the coverage at different position of the reference sequence using the read depth line.

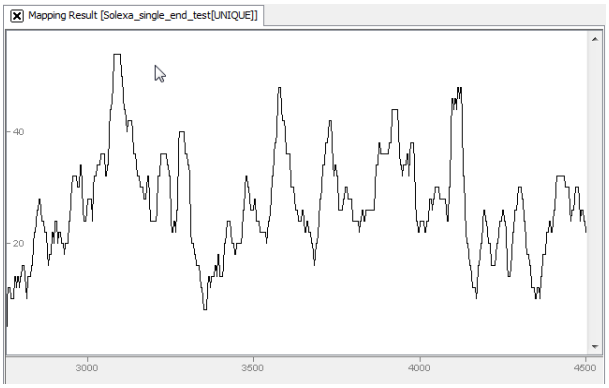
There are several operations allowed on the "mapping results illustrating window":

- Resting the cursor over a position in the "mapping results illustrating window", will bring up a yellow tooltip showing the offset of this position on the reference sequence and the rough coverage of this position.



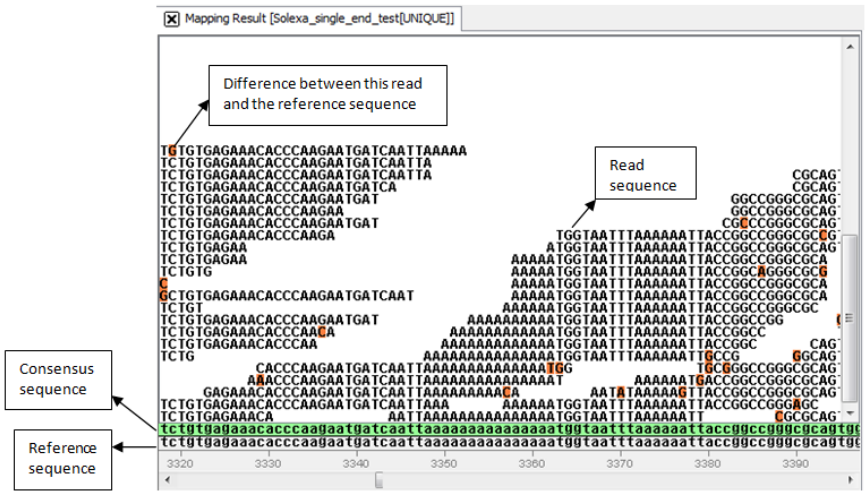
- Scale on the region you are interested in by clicking the left button of the mouse and dragging it into a rectangle, then releasing the left mouse button.





The region in the rectangular region will then be enlarged to the full window of the "Mapping Results Displaying Window" as follows:

- Click on a region that you are interested in or if the length of the focusing region is less than 130bp, the graph will adjust to show the detailed alignments between the reads and the reference sequence.



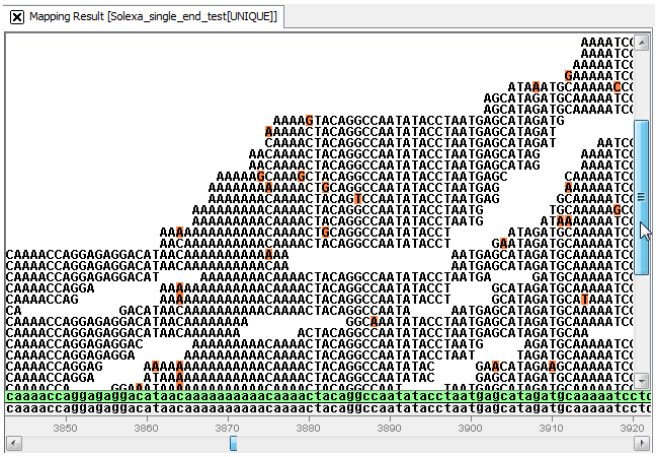
The sequence with a green background is the consensus sequence generated by the mapped reads along the reference sequence. The reads are shown in different scales according to the length of

the region of interest on the reference sequence. A red background of the nucleotides on the read or the consensus sequence highlights a difference from the nucleotide in the same position on the reference sequence.

When clicking on a read, a read rectangle will highlight the read sequence. A blue column will appear highlighting the nucleotides in the same column.

- The horizontal scrollbar acts to increase and decrease the offset of the focus region on the reference sequence.

- The scrollbar on the right helps to observe the reads mapped to this position that do not fit in the window.

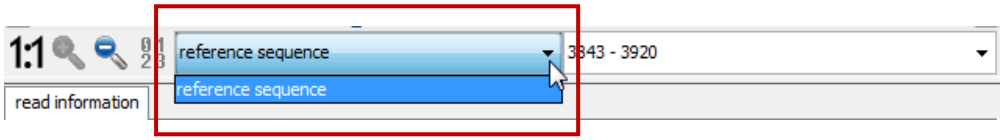


Scaling tools

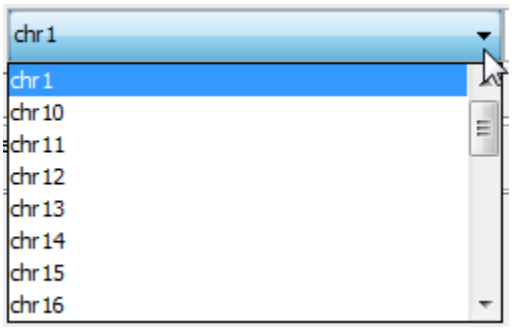
There are three scaling tools which can help you see the mapping results in different scales, .

- : Press this button for the display of the mapping results to go back to the overview of the read depths of all reads mapped to the WHOLE reference sequence.
- : Press this button to display mapping results at a zoomed in rate of 1.2x.
- : Press this button to display the results zoomed out by a factor of 1.2x.

Reference sequence selecting bar



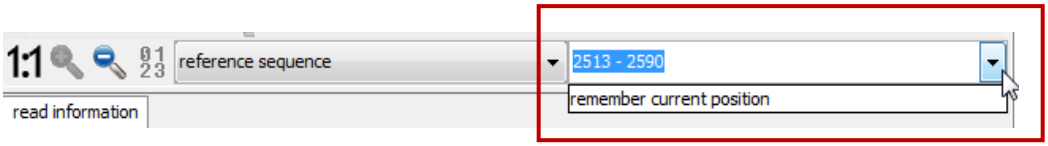
This selecting bar is useful when there are multiple reference sequences.



You can click the dropdown list box to choose a desired reference sequence whose mapping results to be shown. The reads mapped to the selected reference sequence will then be assembled along this reference sequence and displayed. A progress bar will pop up to show the progress.

Reference offset bar

The reference offset bar shows the offset range of the reference sequence shown in the current "mapping results illustrating window".



There are two operations that you can operate on this reference offset bar.

- Locating to a given offset or a offset range

If you want to see the mapping results on a specific position of the reference sequence or a specific range of the reference sequence, type in the offset or the offset range in the locating bar and press the “Enter” key on the keyboard.

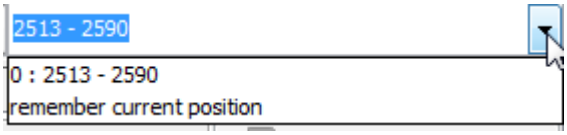
or

The mapping results around this position will then be shown in the “mapping results illustrating window”.

- Remember current position

You can choose to store an offset range and go back to this region afterwards.

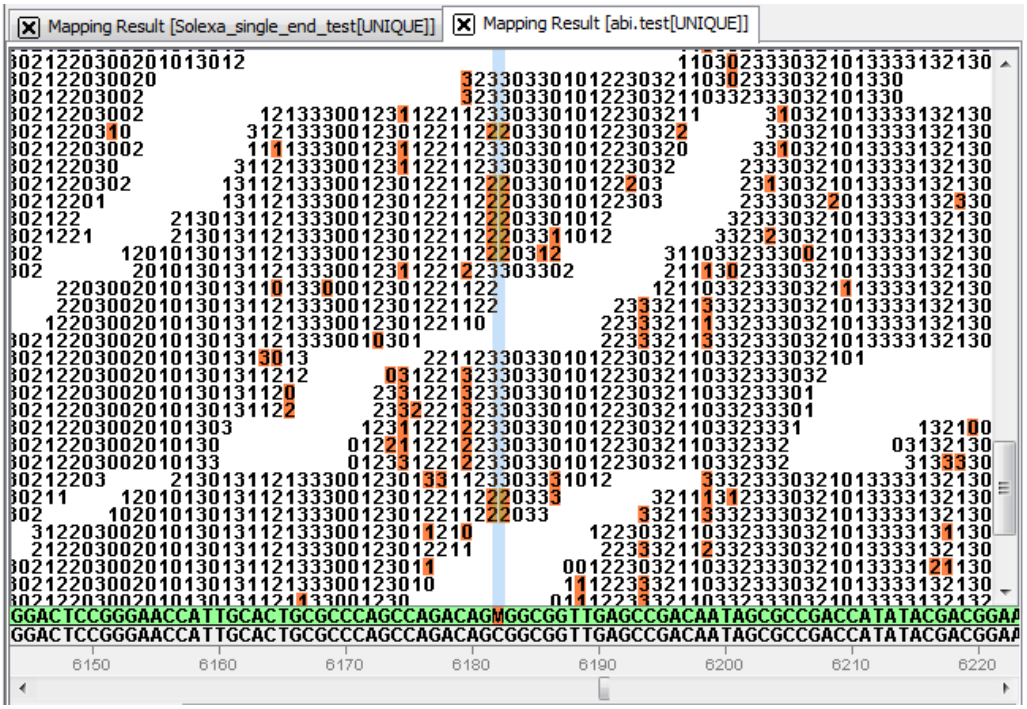
Let us assume that “2513-2500” is our current offset range displayed within the “Mapping Results Illustrating window” on the reference sequence. Click “remember current position” and click the “Reference offset bar” again. You will see that:



“0:2513-2590” is recorded here. You can go back to this region at any time you want.

Switch button

The default display of the read is in nucleotide space (Available only for ABI SOLiD data). For ABI SOLiD data, the default display is the nucleotide reads decoded according to the mapping results. Press the “ ” button to switch the reads display from nucleotide space to color space, or click the “ ” button to go backwards. The reads shown in color space are as follows:



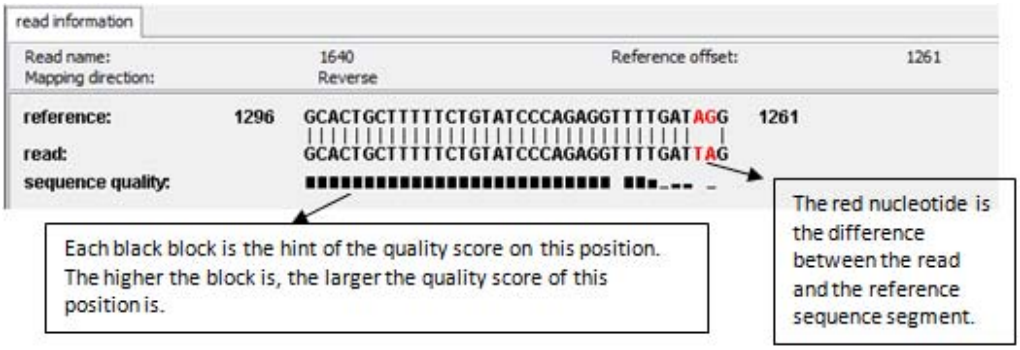
Detailed information panel

This panel is used to show the detailed information in the “Mapping results illustrating window”. Such items as the “Read Information Panel”, “Mapping Summary Panel”, and “SNP panel” are shown on this panel.

- Read information panel

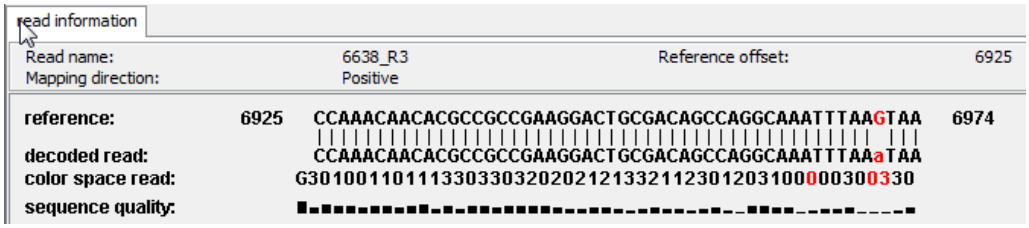
  This panel displays detailed mapping information of a selected reads in the “mapping results illustrating window”.

  Click a read on the “mapping results illustrating window”. The read name, the direction of the mapping, the reference offset where the read is mapped to (the leftmost position) will be shown in the “read base information panel”. The alignment between the target region on the reference and the read will be shown in the alignment panel. If the quality score file is provided when creating the job, the sequencing quality will be illustrated as black blocks. The larger is the length of the block, the higher the quality score of the base.



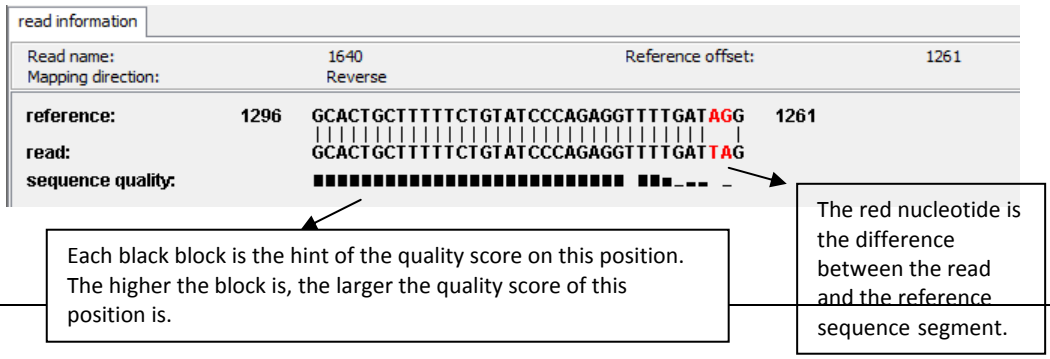
Note that the direction of the alignment is according to the original direction of the read sequence. The start offset and the end offset of the alignment is marked. When the left offset is less than the right offset, the read is mapped to the reverse chain of the reference sequence. The difference between the reference sequence and the read sequence will be marked as red.

For ABI SOLiD reads, both the color space read sequence with the adapter and the nucleotide read sequence decoded from the mapping results by ZOOM will be shown in the following image. Both the sequencing errors on the color space reads and the differences between the decoded reads and the reference sequence will be marked as red.



Clicking “Copy the read sequence” button will copy the read name and the read sequence to the clipboard. If the read is ABI SOLiD data, the color space read sequence will be copied to the clipboard too.

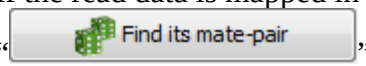
At the same time, more information of this mapped read will be shown in the “read information” tab window below the “Mapping Results Illustrating window”:



Note that the direction of the alignment shown in the “read information” tab is the same as the direction of the read sequence in the read files. If a read is mapped to the reverse chain of the reference sequence, the left offset of the reference segment is larger than the right offset as in the above picture.

Click the “

” button, then the read name and the read sequence will be copied to the clipboard of system.

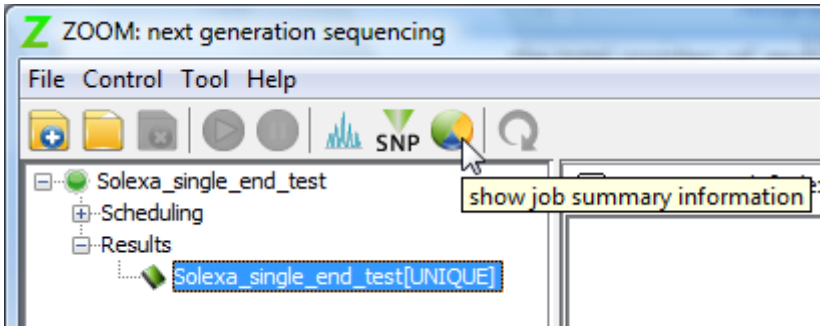
If the read data is mapped in Paired-end / Mate-pair mode, you can select a read and click the “ 

” button. ZOOM will jump to the mate read of the selected reads and the alignment.

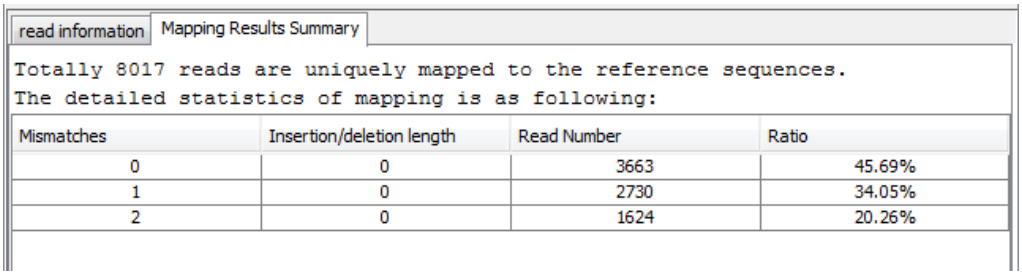
If a read is mapped to multiple positions, press the “<” and “>” button to jump to other positions of this read and show the corresponding alignments.

- Mapping Results Summary panel

Click a [UNIQUE] or [ALL] Result node and click “
” toolbar icon.



The summary of the uniquely mapped results will be show in a “Mapping Summary” tab beside the “read information” tab.


read information
Mapping Results Summary
Totally 8017 reads are uniquely mapped to the reference sequences.
The detailed statistics of mapping is as following:
Mismatches | Insertion/deletion length | Read Number | Ratio
0 | 0 | 3663 | 45.69%
1 | 0 | 2730 | 34.05%
2 | 0 | 1624 | 20.26%

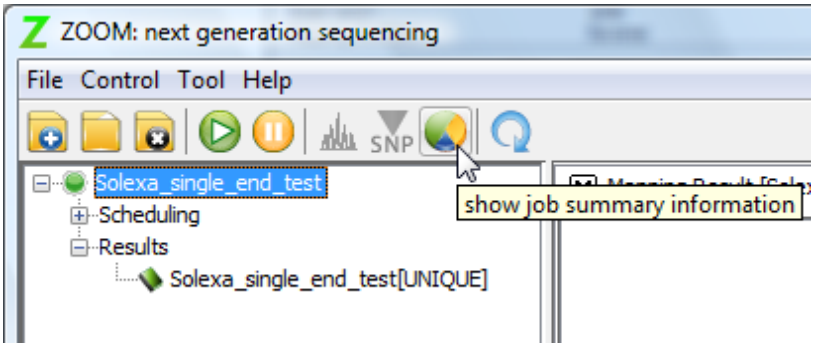
Total number of mapping positions and a statistic table will be shown. The statistic table contains four columns. Each row will show how many mapping positions(the third column) are mapped to the reference sequence with *x* mismatches (the first column) and one Insertion/deletion of length *y* (the second column), and what the ratio of these mapping results over all mapping results (the fourth column).

- SNP panel

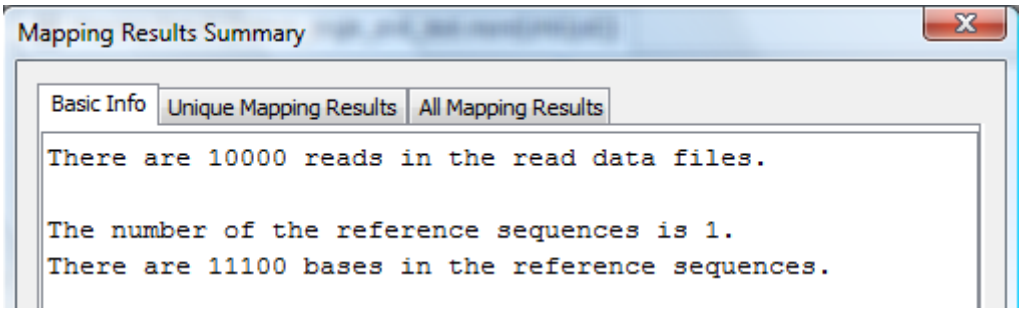
SNP candidates found are listed in a table in the SNP panel, which is shown as a tab window in the "Detailed information panel". Please refer to Section 6.2 in Chapter 6 in this manual for detailed information.

5.2 Show Mapping Results Summary

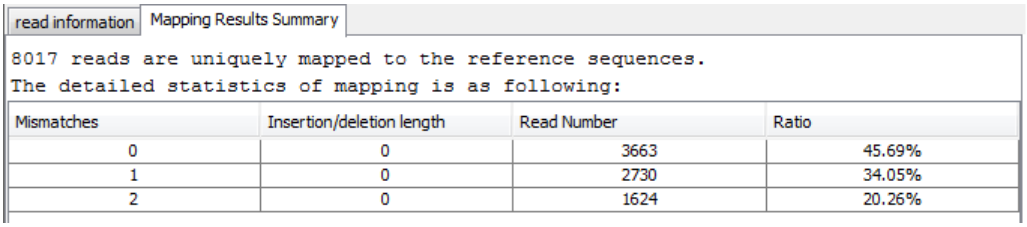
Click a job node and click the mapping results summary toolbar icon " ".



The summary of the mapping results will be shown in the pop up window including: the total number of reads in the read data files; number of reference sequences; the length of the reference sequences;


Mapping Results Summary
Basic Info
Unique Mapping Results
All Mapping Results
There are 10000 reads in the read data files.
The number of the reference sequences is 1.
There are 11100 bases in the reference sequences.

Click on the “Unique Mapping Results” tab will show the number of reads mapped uniquely and the statistics of the uniquely mapping results in the following picture.



If the mapping results of a job include a [ALL] Results node, there will be one “All Mapping Results” tab showing the number of positions mapped in the top N mapping results. Note that the number of the mapped reads is in fact the number of mapping positions, since one read could be mapped to multiple positions since top N mapping results will be kept and output.

```
Totally 2432239 reads are mapped to the reference sequences.
```

Basic Info | Unique Mapping Results | All Mapping Results

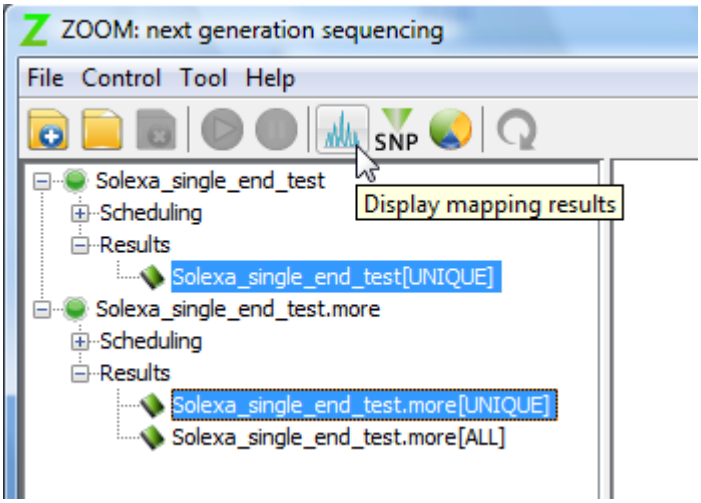
Totally 2432239 reads are mapped to the reference sequences.
The detailed statistics of mapping is as following:

Mismatches	Insertion/deletion length	Read Number	Ratio
0	0	867769	35.68%
1	0	647069	26.6%
2	0	405681	16.68%
3	0	246437	10.13%
4	0	148124	6.09%
0	1	18180	0.75%
1	1	28799	1.18%
2	1	30987	1.27%
3	1	27732	1.14%
0	2	1827	0.08%
1	2	3591	0.15%
2	2	4004	0.16%
0	3	705	0.03%
1	3	1207	0.05%
0	4	127	0.01%

The statistic table contains four columns. Each row will show how many mapping positions(the third column) are mapped to the reference sequence with *x* mismatches (the first column) and one Insertion/deletion of length *y* (the second column), and what the ratio of these mapping results over all mapping results (the fourth column).

5.3 Show Mapping Results Together

If two or more jobs have the same reference sequence, you can choose to merge the mapping results of these jobs to show the mapping results together.



Press “Ctrl” on the keyboard and click the Results nodes you want to show together in the “Job View Panel”, then click the “ ” toolbar icon.

The merged mapping results will be shown in the “mapping results window”. Make sure to select the Results nodes, rather than the tasks. The [UNIQUE] Results node and the [ALL] Results node of one job cannot be selected together. We suggest not select the [UNIQUE] Results node and the [ALL] Results node together even they are from different jobs because showing both uniquely mapped results and top N mapped results might mess up what you really want.

Chapter

6

6. SNP and Small InDels Caller

6.1 Find SNPs and small InDel Candidates

ZOOM builds consensus sequences according to the mapped reads along the reference sequence. If the organism is haploid, there is only one type of nucleotide on each position of the genome. Thus all other nucleotide types of the reads covering this position are caused by sequencing errors or mapping error. ZOOM therefore chooses the majority nucleotide letters of the reads covering this position as the consensus sequence. If the organism is diploid, the nucleotides on the positive chain and the reverse chain could be different. ZOOM adopts a method similar to MAQ[1] to compute the post-probability of each possible genotype and choose the genotype with maximum probability as the consensus sequence. The genotype is coded by the IUPAC code. The mapping relationship of the IUPAC code and the genotype is as follows:

IUPAC code	Genotype of this position
G	<G, G>
A	<A, A>
T	<T, T>
C	<C, C>
R	<G, A> or <A, G>
Y	<T, C> or <C, T>
M	<A, C> or <C, A>
K	<G, T> or <T, G>
S	<G, C> or <C, G>
W	<A, T> or <T, A>

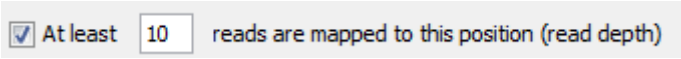
[1] Mapping short DNA sequencing reads and calling variants using mapping quality scores. Li H, Ruan J, Durbin R. *Genome Res.* 2008 Nov;18(11):1851-8.

ZOOM identifies the differences (including mismatches and insertions/deletions) between the consensus sequence and the reference sequence as a primal SNP and InDel candidates set. Note that this version of ZOOM can only find SNPs and short insertions/deletions which occur on read sequences. There are two factors which can affect the confidence of the SNP/InDel candidates:

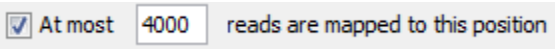
1. the read number covering the position. More reads covering this position means that the position is more likely to be a true variation. However, if the read depth is too high, it might be due to the mapping results of repeated sequences. Thus you can set both the minimal and the maximal read depths.
2. the quality score of a base on a read reflects the probability of whether a base is sequencing error or not. The quality score on the position or the quality scores of the bases around the position affects the probability of whether the difference on the position is a true SNP or not.

According to the above listed factors, ZOOM lists the following five filtering criteria to filter out possible SNPs.

- The requirement of minimal read depth. At least k reads cover this position.

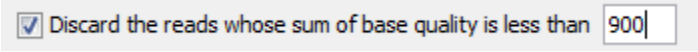


The requirement of maximal read depth. At most k reads are allowed to cover this position.

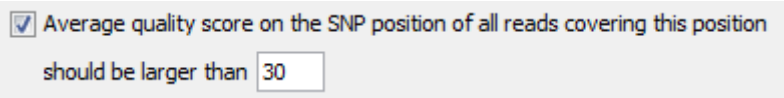


If quality score files are included, quality score can be utilized to filter SNPs:

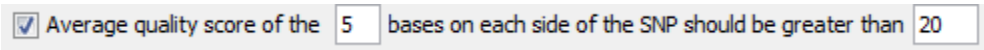
- ZOOM will compute the sum of the quality score of each read sequence, and discard those reads whose sum of base quality score is less than k, because the reads of low quality might be a mapping error.



- The requirement that the variation position should have high quality score, which is measured by the average quality score on the SNP position of all reads covering this position.



- The requirement that the positions around the variation position should be of high quality score.

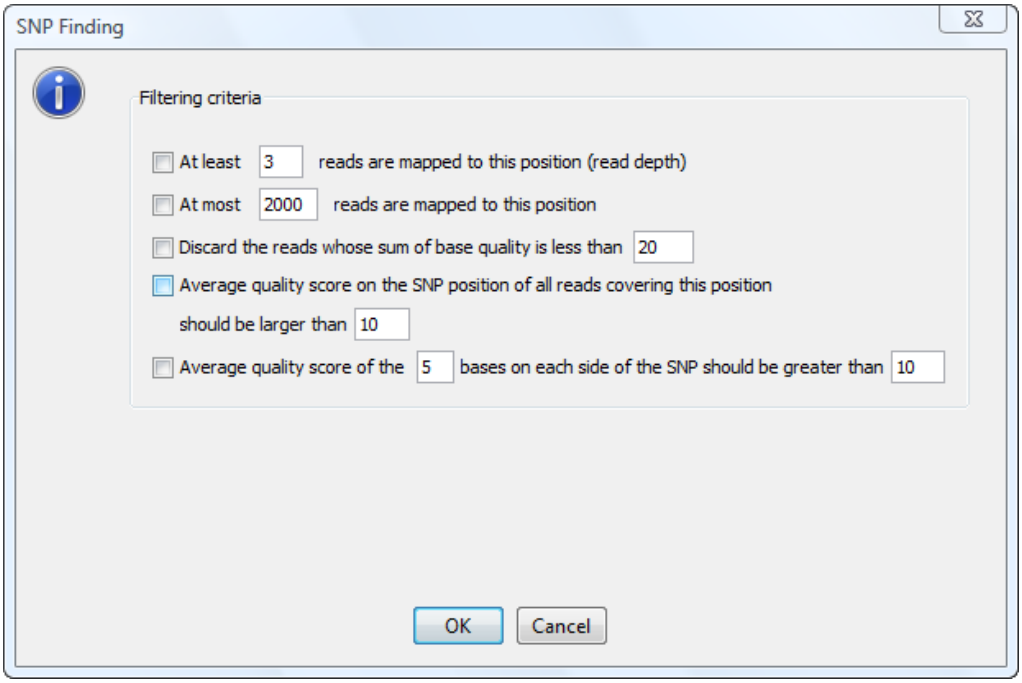


You can choose one or several [UNIQUE] Results nodes to utilize these factors to filter out the SNP candidates satisfying their requirement. Multiple [UNIQUE] Results nodes of jobs with the same reference sequences are allowed to be selected to analyze SNPs together. We suggest the user find SNP candidates only using the uniquely mapped reads. That is to say, only select the [UNIQUE] result nodes rather than [ALL] result nodes to carry out SNP candidate analysis. This is due to the fact that the [All] result node contains top N mapping results for each read, those reads mapped to multiple positions of the reference sequence will make the SNP finding process unreliable.

1. Select one or more [UNIQUE] Results nodes.

2. Click the "filter SNP candidates" toolbar icon "

", or Select "SNP Filter" from the "Tools" menu. A window showing "Filter criteria" will be shown as follows:


SNP Finding
Filtering criteria
At least 3 reads are mapped to this position (read depth)
At most 2000 reads are mapped to this position
Discard the reads whose sum of base quality is less than 20
Average quality score on the SNP position of all reads covering this position
should be larger than 10
Average quality score of the 5 bases on each side of the SNP should be greater than 10
OK
Cancel

3. Click on the checkbox of the filtering criteria which you want to apply towards SNP finding and modify the values in the value fields as you prefer.

4. Press "OK" and the SNP finding will commence on all the reference sequences. A progress bar will pop up. This process may take some time depending on the data size, since all

the reference sequences will be assembled and filtering criteria will be carried out on all the SNP candidates.

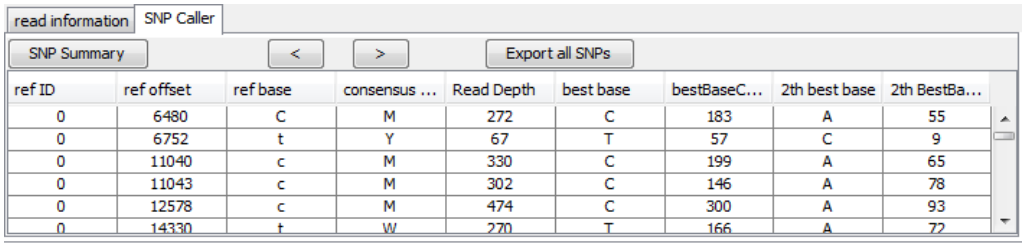
When all SNP candidates are located, a table containing SNP candidates will appear in the "SNP Caller" window.

If you are not satisfied with the SNPs found, press "

" again and try more stringent or less stringent criteria. After this, another tab window entitled "SNP Caller" will be displayed in addition to the existing SNP Caller tab window.

6.2 View SNP Candidates

The "SNP Caller" tab window will show the detailed information of each SNP in a table view as follows:



ref ID	ref offset	ref base	consensus ...	Read Depth	best base	bestBaseC...	2th best base	2th BestBa...
0	6480	C	M	272	C	183	A	55
0	6752	t	Y	67	T	57	C	9
0	11040	c	M	330	C	199	A	65
0	11043	c	M	302	C	146	A	78
0	12578	c	M	474	C	300	A	93
0	14330	t	W	270	T	166	A	72

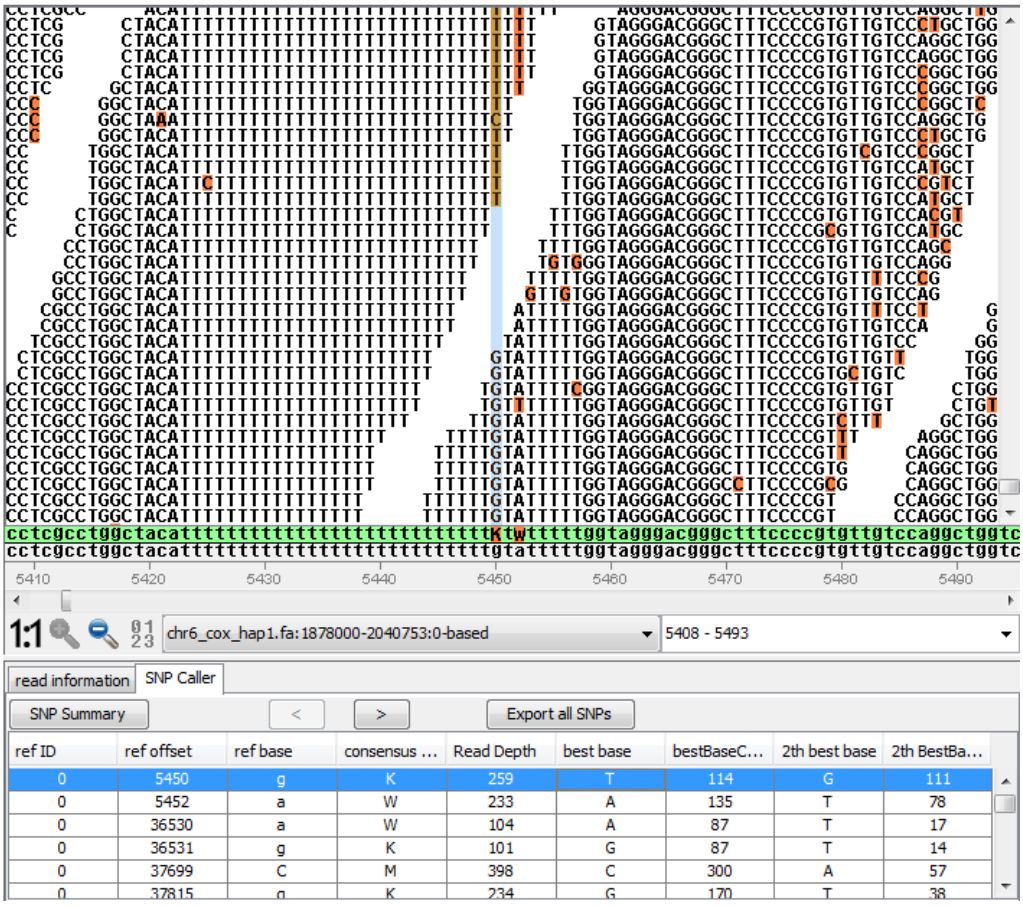
Each row of the table is an SNP candidate. The table has 9 fields showing 9 features of each SNP:

1. refID: the id of the reference sequence, starting from zero
2. ref offset: the offset on the reference sequence where is the SNP located.
3. ref base: the nucleotide of the reference sequence on the position.
4. consensus base: the nucleotide of the consensus sequence on the position. ZOOM can build the consensus sequence in a haploid genome or a diploid genome. If the organism is viewed as a diploid, the nucleotide on the consensus sequence is IUPAC code, which can use one alphabet to denote a haplotype. Say S denotes the haplotype <G , C>, while R means <G, A>.
5. Read Depth: the amount of mapped reads covering the position.
6. best base: the nucleotide with the largest amount of read depth on the SNP position.
7. bestBaseCount: the amount of the best nucleotide on the SNP position.
8. 2nd best base: the nucleotide with the second largest amount on the SNP position.
9. 2nd BestBaseCount: the amount of the second best nucleotide on the SNP position.

" * " is a gap. When it appears in the ref base, then there is an insertion. When " * " is in the consensus base, a deletion occured.

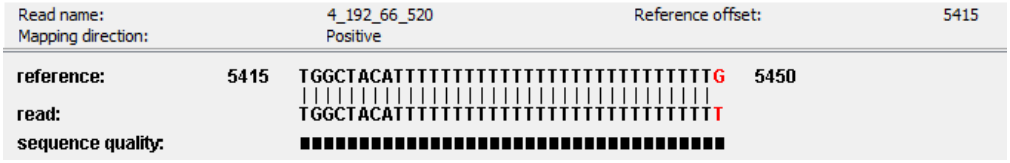
Operations on the Table

- Double click a row in the table. Then the cursor in the "mapping results illustrating window" will jump to the position of this SNP. The column of the position will be highlighted by a blue background.



ref ID	ref offset	ref base	consensus ...	Read Depth	best base	bestBaseC...	2th best base	2th BestBa...
0	5450	g	K	259	T	114	G	111
0	5452	a	W	233	A	135	T	78
0	36530	a	W	104	A	87	T	17
0	36531	g	K	101	G	87	T	14
0	37699	C	M	398	C	300	A	57
0	37815	g	K	234	G	170	T	38

- You can check the bases on this position in detail. Furthermore, you can click the read you are interested in and check the alignment and the quality score of this position in the "read information" panel.

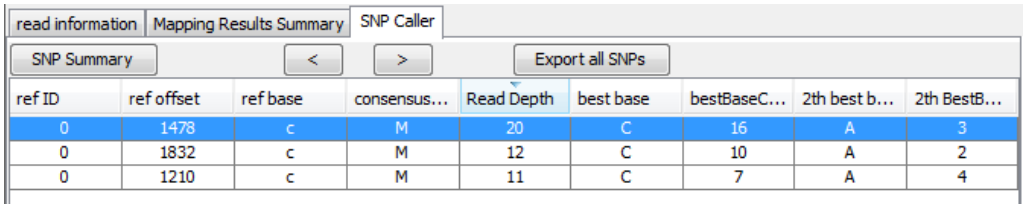


- ">" Forward button and "<" backward button.

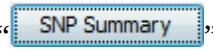
  Press the "<" button or the ">" button. The previous or next row of the SNP will be selected in the table. At the same time, the cursor in the "mapping results illustrating window" will jump to this newly selected SNP.

- Sort the columns in the SNP Table.

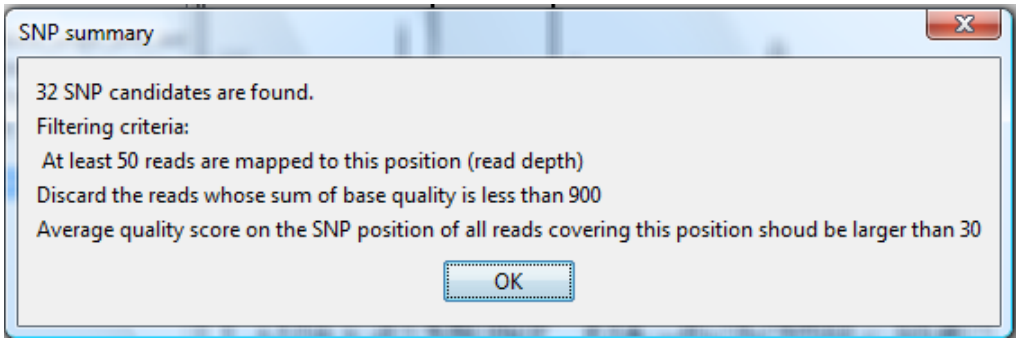
  The nine columns of the SNP Table can be sorted. By default, the table is sorted according to the refId and the reference offset. SNPs with larger read depth might be more reliable. You can choose to sort the SNPs according to the read depth. Click the name of the column and the rows of the table will be sorted according to the contents of this column in ascending order. Click the name once more and the rows will be shown in descending order.



6.3 SNP Summary

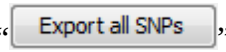
Press the " 

" button in the SNP Caller panel to show the "SNP Summary" window:


SNP summary
32 SNP candidates are found.
Filtering criteria:
At least 50 reads are mapped to this position (read depth)
Discard the reads whose sum of base quality is less than 900
Average quality score on the SNP position of all reads covering this position shoud be larger than 30
OK

The number of SNP candidates and the filtering criteria adopted will be shown.

6.4 Export all SNPs

All SNPs can be exported to a file, by pressing the " 

" button, and choosing a directory and input the file name to store the SNPs. Each line of the file contains the nine fields of one SNP delimited by "tab".

```
<refId> <ref offset> <ref base> <consensus base> <Read Depth>
<best base> <bestBaseCount> <2nd best base> <2nd BestBaseCount>
```

1. refID: the id of the reference sequence, starting from zero
2. ref offset: the offset on the reference sequence where is the SNP located.
3. ref base: the nucleotide of the reference sequence on the position.
4. consensus base: the nucleotide of the consensus sequence on the position. ZOOM can build the consensus sequence in a haploid genome or a diploid genome. If the organism is viewed as a diploid, the nucleotide on the consensus sequence follows the IUPAC code, which uses one letter to denote a haplotype. Say S denotes the haplotype <G, C>, while R means <G, A>.
5. Read Depth: the amount of mapped reads covering the position.
6. best base: the nucleotide with the largest amount on the SNP position.
7. bestBaseCount: the amount of the best nucleotide on the SNP position.
8. 2nd best base: the nucleotide with the second largest amount on the SNP position.
9. 2nd BestBaseCount: the amount of the second best nucleotide on the SNP position.

"*" is a gap. When it appears in the ref base, there is an insertion. When there is "*" in the consensus base, a deletion has occurred.

Chapter

7

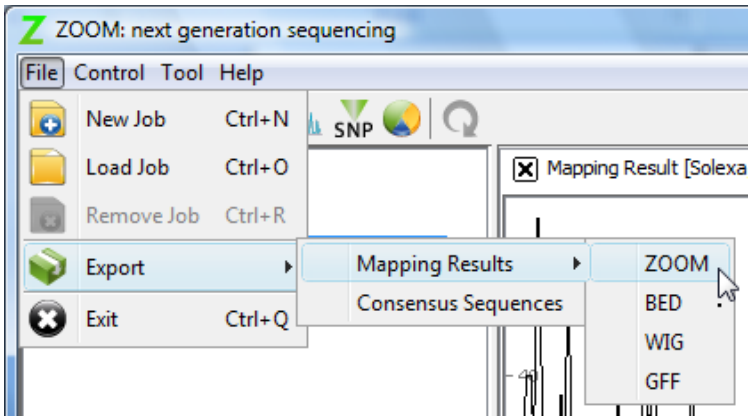
7. Export

ZOOM can export the mapping results, the consensus sequences built and SNP candidates into files. Several commonly used formats are supported to help users to exchange data or get more information in the UCSC genome browser, such as checking whether the alignments fall in the exon regions. The mapping results can be exported into ZOOM format, BED format, GFF format, and WIG format. The consensus sequence can be exported into FASTA format.

7.1 Export Mapping Results

Select the "Results node" of the job that you want to export from the "Job View Panel".

Select "Export" from the "File" menu. Select "Mapping Results" from the popup menu. There are four output formats to export mapping results into.



Select the output directory and output filename from the popup browse window.

ZOOM will output mapping results of the selected results node to the output file. If a [UNIQUE] Results node is selected, the content of the output file is the mapping results of uniquely mapped reads. If an [ALL] Results node is selected, the content of the output file is the top N mapping

results of each read. Note that the mapping results are sorted by the offset of the reference sequences in ascending order. Thus, the top N results of one result might not be listed one by one. The two mates of one pair are not listed one by one either.

ZOOM format

- Output for Illumina/Solexa reads

By default, ZOOM will output the mapping results of each mapped read in the selected Results node.

Each line of the file corresponds to a mapped position, which contains six basic fields delimited by tabs as follows:

```
<Read label> <Reference name> <Reference offset> <Strand>
<Mismatch number> <Insertion/deletion information>
```

If you use "rescore" parameter, which utilizes quality scores to evaluate the mapping probability of the alignment of each mapping result, there is one more field log10 (probability of the alignment).

```
<Read label> <Reference name> <Reference offset> <Strand>
<Mismatch number> <Insertion/deletion information> <Log of mapping
probability>
```

Read label: the name of the mapped read. If there is a tab in the read label, ZOOM will transfer the tab into a space.

Reference name: the name of the reference sequence which this read is mapped to. If there is a tab in the reference name, ZOOM will transfer the tab into the space.

Reference offset: the position that the read mapped on this reference sequence, starting from zero. By default, the leftmost position is always returned, no matter whether the read is mapped to the positive or negative strand.

Strand: the strand of the reference sequence that the read is mapped to. A "+" means the read is mapped to the positive strand of the reference sequence. A "-" means the read is mapped to the negative strand of the reference sequence.

Mismatch number: the Hamming distance, or the number of mismatches between the read and the target region on the reference sequence it maps to.

Insertion/deletion information: the information relating to the insertion/deletion between the read and the target region of the reference sequence on this offset. The field will be the following three cases:

- 'M': No insertion/deletion. Only mismatches found.
- I<length>_<offset>: There is one insertion of length <length> behind <offset>.
- D<length>_<offset>: There is one deletion of length <length> starting from <offset>.

Note that the offset is the offset on the original read sequence, starting from zero, no matter if the read is mapped to the positive chain or the complement chain of the reference sequence.

An example output file:

```
1427   chr6   9      -      1      M

5952   chr6   72     +      0      I2_33
```

How to interpret the results:

- read 1427 is mapped to the offset 9 of the negative strand of chr6 with only one mismatch.
- read 5952 is mapped to the offset 72 of the positive strand of chr6 with zero mismatches and one insertion of length 2 starting at the 34th base of the read.
- read 6353 is mapped to the offset 109 of the negative strand of chr6 with two mismatches and one deletion of length one starting at the 36th base of the read.

Log of mapping probability: log10 (the probability of the alignment). This value is computed using the quality scores of each base. This is a negative number, and the bigger the better.

An example with mapping probability:

```
1427   chr6   9      -      1      M      -6.244083

5952   chr6   9      -      0      I2_33  -1.193035
```

- Output for ABI SOLiD reads

ZOOM can map ABI SOLiD reads within a given Hamming distance, which is the number of mismatches allowed between the read and its target region on the reference sequence. ABI SOLiD reads use the color space format. The differences between the read in color space and the reference sequence are caused either by sequencing error or genomic differences, such as mutations or SNPs. Sequencing errors may cause some reads to be mapped incorrectly to the reference sequence. ZOOM is able to distinguish sequencing errors from genomic differences by correcting the sequencing errors, and allows more reads to be correctly mapped. ZOOM is also able to decode mapped color space reads after error correction and highlight both genomic differences and sequencing errors.

Each line of the file corresponds to a mapped position, which contains eight basic fields delimited by a tab as follows:

```
<Read label> <Reference name> <Reference offset> <Strand> <Total
error number> <Insertion/deletion information> <Decoded nucleotide
sequence> <Mark of sequencing error position>
```

If you use the "rescore" parameter, which utilizes quality scores to evaluate the mapping probability of the alignment of each mapping results, there is one more field ----log10 (probability of the alignment).

```
<Read label> <Reference name> <Reference offset> <Strand> <Total
error number> <Insertion/deletion information> <Decoded nucleotide
sequence> <Mark of sequencing error position> <Log of mapping
probability>
```

Read label: the name of the mapped read. If there is a tab in read label, ZOOM will transfer the tab into a space.

Reference name: the name of the reference sequence which this read is mapped to. If there is tab in the reference name, ZOOM will transfer the tab into a space.

Reference offset: the position that the read mapped on this reference sequence, starting from zero. By default, the leftmost position is always returned, no matter whether the read is mapped to the positive or negative strand.

Strand: the strand of the reference sequence that the read is mapped to. A "+" means the read is mapped to the positive strand of the reference sequence. A "-" means the read is mapped to the negative strand of the reference sequence.

Total error number: Total error number is the summation of the number of mismatches due to genomic differences and sequencing errors of the read. ZOOM will decode the color space read

into nucleotides, in order to separate genomic differences from sequencing errors. The number of the two types of errors will be denoted in the <Decoded nucleotide sequence> and <Mark of positions of sequencing error> fields, respectively.

Insertion / deletion information: the information relating to the insertion/deletion between the read and the target region of the reference sequence on this offset. The field will be the following three cases:

- 'M': No insertion/deletion. Only mismatches found.
- I<length>_<offset>: There is one insertion of length <length> behind <offset>.
- D<length>_<offset>: There is one deletion of length <length> starting from <offset>.

Note that the offset is the offset on the original read sequence, starting from zero, no matter whether the read is mapped to the positive chain or the complement chain of the reference sequence.

Decoded nucleotide sequence: decoded nucleotide sequence of the read after error correction. Genomic differences will be highlighted by lowercase letters. Notice that the first position of the color space read is coded by the first base of the read and the last base of the adapter. ZOOM doesn't include the last adapter base at the beginning of the decoded sequence.

Mark of positions of sequencing errors: This is a binary string which marks the positions of sequencing errors by *"1"*, and the positions without sequencing errors by *"0"*.

An example of <*output*> file:

```
9278   chr1   10   - 1 M ATACGGTTTGAGAATGTAGTCAAACGCCTCCAGTT 00000000000000000000000000000010000
14743  chr1   29   - 2 M CGGTAATACGTGACTCCTGATACGGTTTGAGAATc 00000000000000000000000000001000000000
7222   chr1   32   + 1 I1_33 TCTCAAACCGTATCAGGAGTCACGTATTACCGGAG 00000000000000000000000000000000100
4063   chr1   51   + 1 D1_33 TCACGTATTACCGGGATGCTGCAAAAACTGGAACG 00000000000000000000000000001000000
```

Interpretation of the results:

- read 9278 is mapped to the offset 10 of the negative strand of the reference sequence chr1 with one error and no insertion/deletions, where there is one sequencing error and no genomic difference.
- read 14743 is mapped to the offset 29 of the negative strand of the reference sequence chr1 with two errors, which is the number of polymorphisms on the base space plus sequencing error numbers, and no insertion/deletion. The polymorphism occurs on the last base pair of the nucleotide read while the sequencing error occurs on the 26th base of the color space read.

- read 7222 is mapped to the offset 32 of the positive strand of the chr1, with one error and one insertion of length one starting from the 34th base of the read. The error is a sequencing error on the antepenultimate base of the color space read.
- read 4063 is mapped to the offset 51 of the positive strand of the chr1, with one error and one deletion of length one starting from the 34th base of the read. The error is a sequencing error on count-down 7th base of the color space read.

Log of mapping probability: log10(the probability of the alignment). The value is computed by combining the quality scores of each color space base and the prior probability of SNP occurrence on nucleotide base space. This is a negative number, and the bigger the better. The two values are delimited by a colon.

An example with mapping probability:

```
9278   chr1  10  - 1 M ATACGGTTTGAGAATGTAGTCAAACGCCTCCAGTT 00000000000000000000000000000010000 -3.999175
14743  chr1  29  - 2 M CGGTAATACGTGACTCCTGATACGGTTTGAGAATc 00000000000000000000000000001000000000 -8.596596
7222   chr1  32  + 1 I1_33 TCTCAAACCGTATCAGGAGTCACGTATTACCGGAG 00000000000000000000000000000000100 -3.341088
4063   chr1  51  + 1 D1_33 TCACGTATTACCGGGATGCTGCAAAAACTGGAACG 00000000000000000000000000001000000 -3.532018
```

- Output for paired-end reads data

The output format is same as the output of single-end reads described in the above two sections. The only difference is that the reads in paired-end reads data are mapped in pairs. Each read of a pair is mapped to the reference sequence within the allowed mismatches or edit distances, as is done for the single-end read case. The user needs to judge whether the pair is a correct mate-pair, has an insertion/deletion or is a translocation according to the strand and the offset where they are mapped to.

BED format

BED format provides a flexible way to define the data lines that are displayed in an annotation track of the UCSC browser. The BED file is used to show the alignments between the reads and the reference sequences.

If there are several reference sequences, each BED file may have several tracks. Each track shows the read alignments in this reference sequence.

Each BED track has one annotation line on the heading of the file describing the features of this file and the configuration needed to show the results in UCSC genome browser. You can revise the head of the file to get the display effect as you like.

```
track name="Reads Alignments on Chr1" description="Reads alignments
show" visibility=2 itemRgb="On" useScore=0
```

The mapping results will be shown line by line, which are described by nine BED fields in each line of the file with the tab delimited.

1. **chrom** – The name of the chromosomes (e.g. chr3, chrY, chr2_random), which is the names described in the reference sequence files. Thus if you want the BED file be shown correctly in the UCSC genome browser, please make sure that the reference names in the reference sequence files are accepted in the UCSC genome browser.
2. **chromStart** – The starting position of the alignment in this reference sequence. The first base in a chromosome is numbered as 0.
3. **chromEnd** – The ending position of the alignment in this reference sequence. The *chromEnd* base is not included in the display of the alignment. For example, an alignment defined as *chromStart*=0, *chromEnd*=50, spans the bases numbered 0-49.
4. **read name** – The name of the read.
5. **score** -- A score between 0 and 1000. We use this item to store the edit distances between the read and the reference sequence, i.e. the addition of the mismatch number and the length of the insertion/deletion.
6. **strand** -- The mapping direction of the read. '+' means the read is mapped to the positive chain of the reference sequence, while '-' means the read is mapped to the reverse chain.
7. same as 2
8. same as 3
9. **itemRgb** – An RGB value of the form R, G, B (eg. 255,0,0). If the track line itemRgb attribute is set to “On”, this RBG value will determine the display color of the data contained in this BED line. If item 6 is '+', the item is "255,0,0". If item 6 is '-', the item is "0,0,255". In this way, the read mapped to the positive chain will be shown in the color red. The read mapped to the reverse chain will be shown in the color blue.

Here is an example of a BED file containing mapping results on two chromosomes:

```
track  name="Reads  Alignments  on  chr7"  description="Reads  alignments  show"
       visibility=2 itemRgb="On"

chr7   127471196  127472363  4_87_829_866  0  +  127471196  127472363  255,0,0

chr7   127472363  127473530  4_87_923_316  0  +  127472363  127473530  255,0,0

chr7   127473530  127474697  4_87_239_596  0  +  127473530  127474697  255,0,0

chr7   127474697  127475864  4_87_199_751  0  +  127474697  127475864  255,0,0

chr7   127475864  127477031  4_87_345_944  0  -  127475864  127477031  0,0,255

chr7   127477031  127478198  4_87_863_562  0  -  127477031  127478198  0,0,255

chr7   127478198  127479365  4_87_810_633  0  -  127478198  127479365  0,0,255

chr7   127479365  127480532  4_87_647_665  0  +  127479365  127480532  255,0,0

chr7   127480532  127481699  4_87_872_656 0  -  127480532  127481699 0,0,255




track  name="Reads  Alignments  on  chrY"  description="Reads  alignments  show"
       visibility=2 itemRgb="On"
```

For more information about the BED format, please refer to the website of UCSC. http://genome.ucsc.edu/FAQ/FAQformat#format1.

GFF format

GFF (General Feature Format) lines are based on the GFF standard file format. GFF lines have nine required fields that *must* be tab-separated. If the fields are separated by spaces instead of tabs, the track will not display correctly. For more information on GFF format, refer to http://www.sanger.ac.uk/Software/formats/GFF.

1. **seqname** - The name of the reference sequence. Must be a chromosome or scaffold.
2. **source** - The data source. This field is “solexa” or “solid” according to the data type.
3. **feature** – The name of the read

4. **start** - The starting position of the alignment in the reference sequence. The first base is numbered 1.
5. **end** - The ending position of the alignment in the reference sequence. This *end* is included in the display of the alignment. For example, an alignment defined as start=1, end=50, spans the bases numbered 1-50.
6. **score** - A score between 0 and 1000. We use this item to store the edit distances between the read and the reference sequence, i.e. the addition of the mismatch number and the length of the insertion/deletion.
7. **strand** - The mapping direction of the read. '+' means the read is mapped to the positive chain of the reference sequence, while '-' means the read is mapped to the reverse chain.
8. **frame** - ZOOM set this field to be '.'

Example:

```
track name="Reads Alignment on DH10B" \

description="Reads alignments show"

DH10B  solid  2852_R3      13     63     2      +      .

DH10B  solid  4085_R3      13     63     0      +      .

DH10B  solid  7489_R3      13     63     1      +      .
```

For information about GFF format, please refer to

http://www.sanger.ac.uk/Software/formats/GFF/GFF_Spec.shtml

WIG format

The wiggle (WIG) format is for display of dense, continuous data such as transcriptome data. ZOOM uses this format to store the coverage (read depth) of each genome position in the selected regions.

Each WIG file has several annotation lines on the head of the file describing the features of this file and the configuration about how to show the results in UCSC genome browser. You can revise the head of the file to get the display effect as desired.

```
track type=bedGraph name="Bed Format" description="BED format"
visibility=full color=200,100,0 altColor=0,100,200 priority=20
```

The mapping results will then be shown line by line, which are described by nine BED graph fields in each line of the file with tab delimited. We adopt the BED Graph format with four fields since there could be different genomes in the reference sequences.

1. chrom: The name of the reference sequence
2. chromStartA: The offset of the position, start from zero
3. chromEndA: The offset of the position, the same as chromStartA
4. dataValue: The coverage / read depth of this position

WIG file format Example:

```
track type=bedGraph name="Bed Format" description="BED format"
visibility=full color=200,100,0 altColor=0,100,200 priority=20

chr1    556776  556776  50

chr1    556777  556777  50

chr1    556778  556778  50

chr1    556779  556779  50

chr1    556780  556780  50

chr1    556781  556781  38

chr1    556782  556782  38

chr1    556783  556783  38

chr1    556784  556784  38
```

The description of WIG format is on the website of UCSC

(http://genome.ucsc.edu/goldenPath/help/wiggle.html).

7.2 Export Assembled Consensus Sequence

Select a [UNIQUE] Results node of a job or several [UNIQUE] Results nodes of jobs with the same reference sequences.

Select “Export” from the “File” menu. Select “Assembled Sequences” from the popup menu. The assembled consensus sequence built according to the mapping result will be exported in FASTA format. There are two ways to export the assembled consensus sequence:

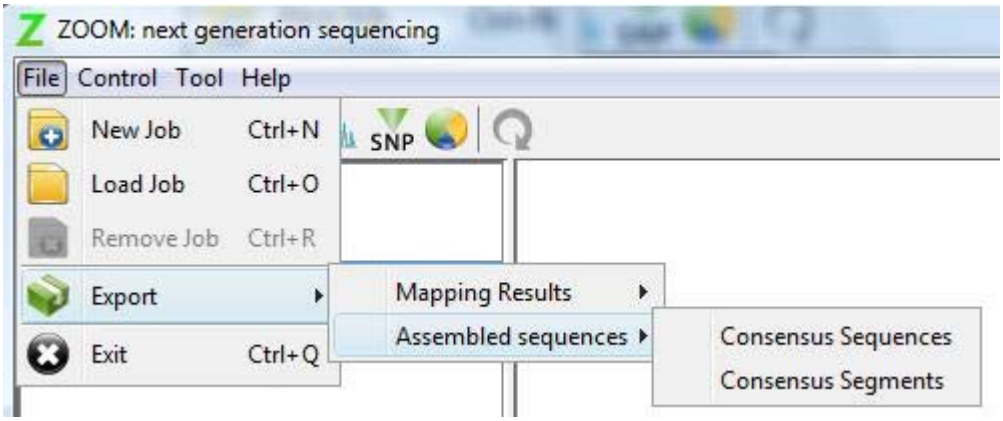
- Consensus sequences
- Consensus segments

The difference is that:

if you choose “Consensus sequences”, one reference sequence will export one assembled consensus sequence. Those bases with no reads covering will be denoted by “.”.

If you choose “Consensus segments”, the consensus sequence of one reference sequence may be exported in several segments separated by the “gap” regions where no reads cover.

Several jobs with the same reference sequence can be selected together to output one consensus sequence. Note that we suggest only building consensus sequences on the [UNIQUE] result nodes. Because the [All] result node contain top N mapping results for each read, those reads mapped to multiple positions of the reference sequence will make the SNP finding process unreliable.



Consensus sequence in FASTA format

The output file contains the assembly of mapped reads along the reference sequence. If multiple reference sequences are used, multiple consensus sequences will be output in multi-FASTA

format in one file. If there are some bases without any reads mapped to, these positions on the consensus sequence are denoted by ".".

When the organism is selected to be treated as diploid genome, the nucleotides on the positive chain and the reverse chain could be different. ZOOM adopts a way similar to MAQ to compute the post-probability of each possible genotype and chooses the genotype with maximal probability as the consensus sequence. The genotype is coded by the IUPAC code.

The mapping relationship of the IUPAC code and the genotype is as follows:

IUPAC code	Genotype of this position
G	<G, G>
A	<A, A>
T	<T, T>
C	<C, C>
R	<G, A> or <A, G>
Y	<T, C> or <C, T>
M	<A, C> or <C, A>
K	<G, T> or <T, G>
S	<G, C> or <C, G>
W	<A, T> or <T, A>

When the organism is selected to be treated as haploid genome, the assembly process constructs a consensus sequence using the following major vote process:

If [#deletion] > [#A+#C+#G+#T+#N], then there is a deletion at this position, otherwise the nucleotide with the highest frequency will be chosen. If the read coverage is less than <*mincov*>, the letter is lowercase (unreliable base), otherwise it is uppercase (reliable).

If [#insertion] > [#continuous] (the number of reads which do not agree that there should be an insertion), then there is an insertion after this position, and the sequence segment with the highest frequency (collected from reads) will be inserted into the consensus sequence.

Consensus segments in FASTA format

The output file contains the assembly of mapped reads along the reference sequence. Since it is probable that no reads are mapped to some regions of the reference sequence, there are gaps in the assembly. ZOOM can export the assembly sequence as several segments separated by gaps of length that you prefer. This option is quite useful in some applications such as RNA-seq.

After you choose to export the “assembly sequence” in “Consensus segments”, a window will pop up asking you to enter the minimal gap size. ZOOM will split the sequence with gaps larger than the minimal gap size into two segments.

Each segment will in the following format:

```
><reference sequence name>_Contig_<the number of the current segment>
StartPos <the offset of the start position of this segment>

the segment sequence
```

Here is an example:

```
> gi|224384768|gb|CM000663.1| Homo sapiens chromosome 1_Contig_15
StartPos 11234

TACGTAGCTTGAACAAAAACCTCGATG
```

Chapter

8

8. 100% sensitivity cases

This chapter lists all cases where the current release of ZOOM can guarantee 100% sensitivity. ZOOM designs a framework to construct the efficient spaced seeds sets which can achieve 100% sensitivity for a large range of read lengths and mismatch numbers. These spaced seeds sets guarantee great accuracy and speed of ZOOM. The cases that guarantee 100% sensitivity in this release are listed in the following table. For cases with more mismatch numbers and cases with insertion/deletion, ZOOM still has good sensitivity. If you do require 100% sensitivity beyond the listed cases, please contact us. We will be happy to design seeds specifically for your requirement.

8.1 Cases for Illumina/Solexa data

Read Length Range (bp)	Mismatch Numbers
12-256	No more than 0
14-250	No more than 2
25-246	No more than 3
30-236	No more than 4
78-191	No more than 5
91-178	No more than 6

8.2 Cases for AB SOLiD data

Since the data format of ABI SOLiD is color space, ZOOM extends the multiple spaced seeds set used for Illumina/Solexa data. Spaced seeds are used between the color space of reads and the color space of reference sequences. Note that in the following table, the mismatch number is the summation of the polymorphism number on base space and the sequencing error number on color space. However, since one polymorphism occurs on base space, there are two adjacent mismatches on the color space. So the mismatch number on the color space is in fact at most the summation of sequencing error number on color space and two times the polymorphism number on base space.

For example, if a read of length 50bp has four polymorphisms with its target region on the reference sequence, there are at most eight mismatches between the color space of this read and its target region.

For example, for reads of length 35 bp, ZOOM will find all the mapping results which have:

- Three polymorphisms on the base space (in total, six mismatches between the color space of reads and the reference sequence).
- Two polymorphisms on the base space and one sequencing error on the color space (in total, five mismatches between the color space of reads and the reference sequence).
- One polymorphism on the base space and two sequencing errors on the color space (in total, four mismatches between the color space of reads and the reference sequence).
- Zero polymorphisms on the base space and three sequencing errors on the color space. (in total, three mismatches between the color space of reads and the reference sequence)

Read Length range (bp)	Mismatch numbers
24-244	No more than 1
25-255	No more than 2
30-242	No more than 3
42-228	No more than 4

5.

Chapter

9

9. Frequently Asked Questions

Question: Can I put reads of different lengths in the same file?

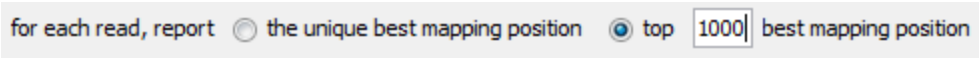
Answer: Yes, ZOOM will automatically call different parameter sets for different read lengths, and the results will be merged

Question: Is the input read data case sensitive?

Answer: No, “a”= “A”, “c”= “C”, “g”= “G”, “t”= “T”= “u”= “U”, and all other letters are “N”. If you have different requirements, please contact us.

Question: Can I get all mapped positions for each read, in addition to the uniquely mapped information?

Answer: Yes, set the parameter in the “collecting results” part as follows to output the top N best mapping results for each read. Set N very large if you want all mapping results for each read.



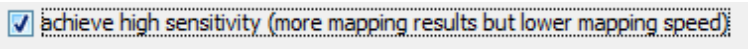
Question: In which cases can ZOOM achieve 100% sensitivity?

Answer: ZOOM designs a framework to construct the efficient spaced seeds sets which can achieve 100% sensitivity for a large range of read lengths and mismatch numbers. All cases in this release are listed in Chapter 8. For cases with more mismatch numbers and cases with

insertion/deletion, ZOOM also has good sensitivity. If you do need 100% sensitivity beyond the listed cases, please contact us.

Question: How do I get better sensitivity using 100% sensitivity seeds without too much running time?

Answer: Run ZOOM without setting the "achieve high sensitivity" option first. Then extract the unmapped reads to run with ZOOM by clicking the "achieve high sensitivity" option.

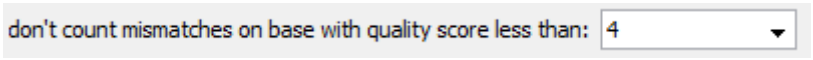


Question: Can ZOOM find short indels?

Answer: Yes. However, ZOOM can only find one gap with any length on the read sequence. The speed is about five times slower than the mode that only allows mismatches when each indel is allowed.

Question: The quality of 3'-end reads is not very good, what should I do?

Answer: You can set a threshold between high quality bases and low quality bases. Check the box and modify the threshold in the following text field:



ZOOM will neglect those low quality bases when mapping.

Question: How many reads can ZOOM deal with in 8G RAM?

Answer: For command-line version, we suggest 25~30 million reads for 8G RAM. If you double your RAM doubles, you can also double the data size. For GUI version, ZOOM can split reads into small pieces. You can modify the size of the small pieces to run on different size of RAM. Please refer to Section 2.6.

Question: Can ZOOM schedule multiple jobs on multiple CPUs of one server or multiple servers?

Answer: Yes. Please configure the server address using the Configuration button " " on toolbar, and ZOOM will split the job into several tasks, schedule among these servers and collect the mapping results automatically. You can also choose the data size of each task running on each CPU according to the RAM of your server.

Question: Can ZOOM utilize the quality score of reads to enhance mapping results?

Answer: Yes. For Illumina/Solexa data, ZOOM adopts two ways to utilize quality scores to enhance mapping results. The first way is to only count mismatches occurring on high quality positions. The second is to utilize quality score of each base of the read to compute the mapping probability of possible alignments for each read and choose the best or top N mapping results according to the mapping probability. For ABI SOLiD data, because ZOOM can differentiate possible genomic differences from sequencing errors on color space, ZOOM computes the mapping probability of alignments for each read utilizing both quality scores and the probability of an SNP occurring in the organism you sequenced. Then it uses the mapping probability to assess and choose the best or top N mapping results for each read. Please refer to Section 4.3 for more information.

Question: Can ZOOM output the SNP candidates or the INDEL variation candidates?

Answer: Yes. You can ask ZOOM to carry out the post-analysis to find SNP candidates and view them in an intuitive way.

Question: Can ZOOM output the structural variation according to the output of paired-end mapping?

Answer: Not yet. In this release, ZOOM outputs those read pairs mapped in the distance range. You should judge whether there is structural variation by the mapping offsets and direction of the two mates of one pair. ZOOM offers the "process unmapped reads" function which will map

those unmapped reads with a different distance range or map them in single-end mode. This might help you to identify structural variation.

Question: Are there restrictions on the length of reads label?

Answer: No. However, please don't use spaces inside the label for the 'one read per line' format, because spaces aid in identifying where the read data field begins.

Question: Can ZOOM deal with 454 data or Helicos data?

Answer: ZOOM is optimized for Illumina/Solexa and ABI SOLiD data. ZOOM can get good mapping results on these two instruments. However, the sequencing error types of 454 instrument and Helicos instrument are quite different, which contain many short indels. ZOOM can't guarantee good mapping results because currently ZOOM can only handle one gap of any length rather than many gaps. However, you could give it a try could since ZOOM can handle reads over 200bp and can deal with reads of variable lengths automatically. Any feedback is appreciated. We would like to support these two instruments in the future.

10. About BioinformaticsSolutions Inc.

BSI *provides advanced software tools for analysis of biological data.*

Bioinformatics Solutions Inc. develops advanced algorithms based on innovative ideas and research, providing solutions to fundamental bioinformatics problems. This small, adaptable group is committed to serving the needs of pharmaceutical, biotechnological and academic scientists and to the progression of drug discovery research. The company, founded in 2000 in Waterloo, Canada, comprises a select group of talented, award-winning developers, scientists and sales people.

At BSI, groundbreaking research and customer focus go hand in hand on our journey towards excellent software solutions. We value an intellectual space that fosters learning and an understanding of current scientific knowledge. With an understanding of theory, we can focus our talents on providing solutions to difficult, otherwise unsolved problems that have resulted in research bottlenecks. At BSI, we are not satisfied with a solution that goes only partway to solving these problems; our solutions must offer something more than existing software.

The BSI team recognizes that real people will use our software tools. As such, we hold in principle that it is not enough to develop solely on theory; we must develop with customer needs in mind. We believe the only solution is one that incorporates quality and timely results, a satisfying product experience, customer support and two-way communication. So then, we value market research, development flexibility and company-wide collaboration, evolving our offerings to match the market/user's needs.

Efficient and concentrated research, development, customer focus and market analysis have produced: PEAKS software for protein and peptide identification from tandem mass spectrometry data, RAPTOR for threading based 3D protein structure prediction, PatternHunter software for all types of homology search sequence comparison and ZOOM for next generation sequencing.

11. ZOOM Software License

This is the same agreement presented on installation. It is provided here for reference only.

If we are evaluating a time limited trial version of ZOOM and we wish to update the software to the full version, we must purchase ZOOM and obtain a full version registration key.

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12. Reference: ZOOM Paper

Please use the following references when publishing a study that involved the usage of ZOOM.

ZOOM! Zillions of Oligos Mapped. Hao Lin, Zefeng Zhang, Michael Q. Zhang, Bin Ma, and Ming Li. *Bioinformatics* 2008 24(21):2431-2437

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